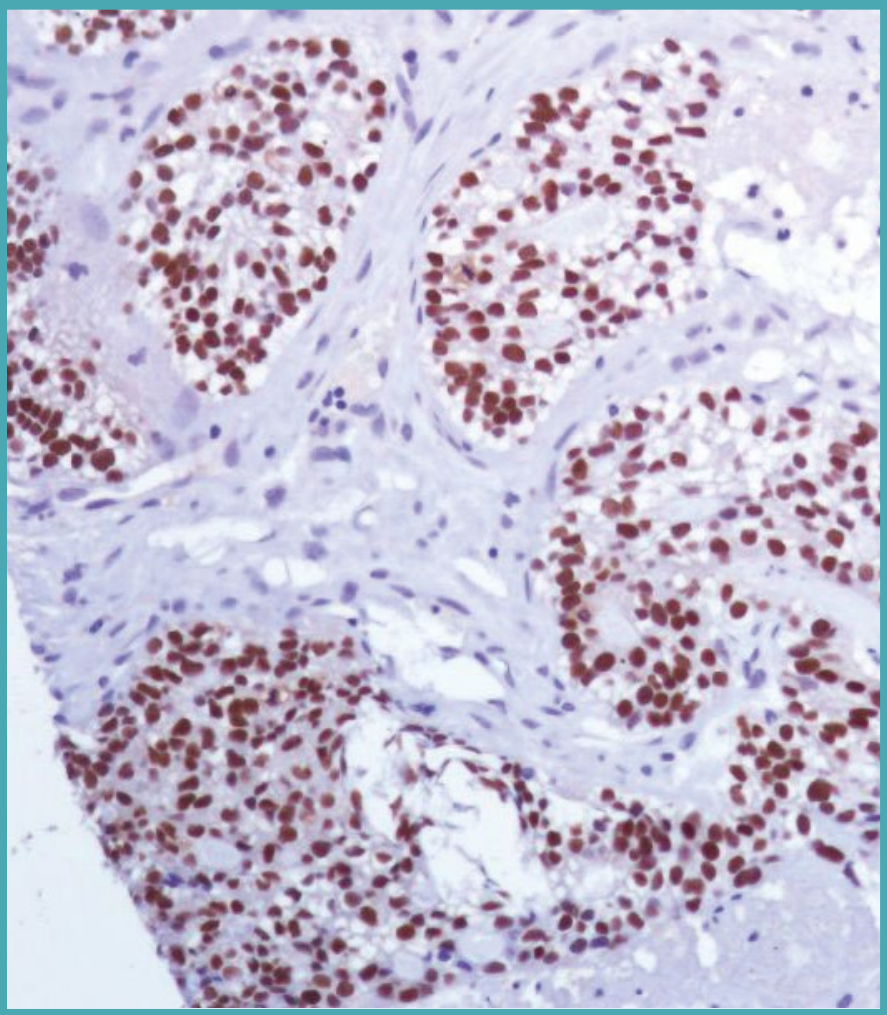
cancer biomarkers guide

to help you progress faster



progress happens together

abcam

Contents

Immunohistochemistry in cancer biomarker research

The impact of cancer biomarkers on the progression and implementation of cancer research has been vast. Cancer biomarkers play a pivotal role in research, from identifying new biomarkers for improved diagnostics and drug development to validating biomarkers across cancer types and stages or prioritizing targets for translation to preclinical research. Biomarkers have become central to targeted drug development and patient segmentation, with clinical trials incorporating biomarkers being more likely to succeed than those without biomarkers[1]. Continuing research in this field offers hope for new biomarkers and increased validation and applications of current biomarkers to improve diagnostics and therapeutics for all cancer types.

IHC is a hugely popular tool in cancer research and remains the gold standard in clinical cancer diagnostics. Each type of cancer is associated with specific protein biomarkers that can be identified via IHC to offer a powerful tool for investigating tumor development and responses. Using this technique, researchers can identify the histogenesis of tumors, stage tumors, predict tumor prognosis, and predict and monitor therapeutic response. Knowing the utilities and pitfalls of each tumor-associated biomarker is essential to avoid potential diagnostic errors, as no absolutely cancer-specific biomarker exists.

These tests can be run quickly, easily, and at a low cost compared to other diagnostic methods. IHC is thus a robust and reliable technique and, with a good panel of specific biomarkers, is ideal for rapid clinical diagnosis.

Choosing an antibody for IHC

Whatever cancer type you are interested in, an essential starting point is the use of a sensitive, specific, and consistent IHC antibody. Antibody validation is key to ensuring antibody specificity and reproducibility. We use a variety of experimental applications to validate our antibodies, including IHC, and many of the antibodies on our catalog will state that they are suitable for use in IHC.

For consistent results between your IHC experiments and to ensure the long-term viability of your research, we recommend using an antibody generated using recombinant technology (i.e. recombinant antibodies). These antibodies are developed *in vitro* and do not rely on an animal's immune system for production, meaning that batch-to-batch, they deliver consistency of performance.

In this guide, we have included a number of the most common cancer types: breast, lung, colorectal, prostate, ovarian, and pancreatic cancer, and a range of emerging and approved biomarkers for diagnosis, prognosis and therapeutic monitoring.

Here you can find the right biomarker for your research and select the most highly recommended antibody for use in IHC.

References

1. Wong CH, Siah KW, Lo AW. Estimation of clinical trial success rates and related parameters. *Biostatistics*. **20**(2):273-286 (2019).

Biomarkers of cancer metastasis

ABL1	PIK3CD
CDH1	ROCK1
FOS	STAT3
MMP2	PEG10
PAK4	FAT1

Metastasis involves the spread of cancer from the primary tumor to distant tissues and organs. This process is a significant factor in the determination of cancer morbidity and a characteristic of cancer malignancy.

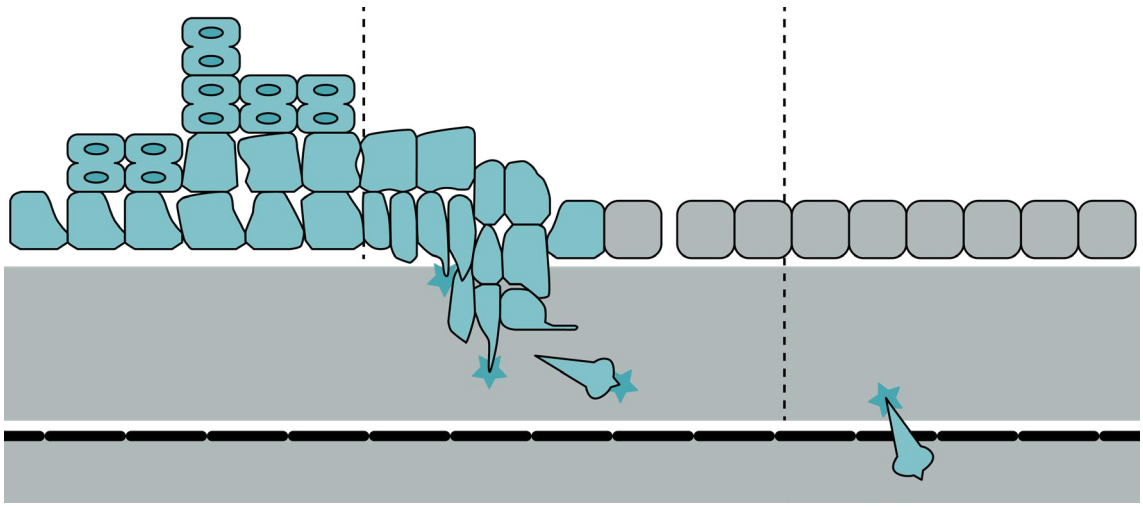
IHC has become an important application for identifying and classifying carcinomas of unknown primary site[1]. The prediction of the metastatic potential of a tumor is important and could allow for personalized therapy at an early stage to better treat cancers. Accurate biomarkers of metastasis thus represent an enormous advance in the potential clinical treatment of cancer. The use of these cancer biomarkers in the clinic could help to detect the initial stages of metastasis, preferred sites of cancer metastasis and the probability of cancer recurrence.

The metastatic cascade can be separated into three main processes:

— Invasion occurs as tumor cells acquire the ability to penetrate surrounding tissues, passing through the basement membrane and extracellular matrix.

— Intravasation involves the penetration of the lymphatic or vascular system by the motile tumor cells.

— Extravasation involves the journey of metastatic cancer cells through the circulatory system to invade the vascular basement membrane and extracellular matrix at a secondary site.



Extracellular matrix changes

To dissociate from the primary tumor mass and invade the surrounding stroma, metastatic cancer cells must undergo the loss of cell-cell adhesion and changes in cell-matrix interactions[2]. These changes include the secretion of heparanases and matrix metalloproteinases to degrade the basement membrane and extracellular matrix (ECM) and the expression/suppression of proteins involved in motility and migration.

Many metastatic biomarkers have been associated with the dysregulation of the ECM to promote metastasis. The tight junction transmembrane protein Claudin-7 is utilized as a prognostic biomarker in the analysis of invasive ductal breast cancer and is being explored as a cancer biomarker to distinguish chromophobe renal cell carcinoma from other renal tumor subtypes[3].

Claudin-1 expression is used as a prognostic biomarker and potential drug target for lung adenocarcinoma cases[4]. E-Cadherin and Vimentin have been described as prognostic biomarkers for patients with non-small cell lung cancer treated with erlotinib[5].

Epithelial to mesenchymal transition

Epithelial to mesenchymal transition (EMT) is a staged process where epithelial cells lose cell polarity and adhesion and transition to an invasive, metastatic cell phenotype. This transition is governed by a number of growth factors and signaling pathways, including TGFβ, Wnt, and Notch. These signaling pathways show cross-talk that facilitates EMT and subsequent cell invasion.

Various additional factors have been shown to contribute to EMT, such as hypoxia, metabolic stressors, matrix stiffness and epigenetic and post-translational modulators. The precise contribution of each of these factors to EMT remains unknown and may vary according to each specific cancer[6].

A number of biomarkers for the EMT process are used clinically in the diagnosis and prognosis of various cancer types. Downregulation of E-cadherin is a key event in EMT, and the relationship between E-cadherin loss and EMT has been established in many cancer types[7].

Mucins are secreted from and localized to the apical borders of healthy epithelial cell sheets. Over-expression of MUC1, MUC4, and MUC16 are all utilized in the diagnostic and prognostic assessment of cancers, including breast, pancreatic and ovarian cancers[8].

Angiogenesis and lymphangiogenesis

The tumor must initialize angiogenesis, without which it would fail to develop, as local diffusion for the transport of nutrients to and removal of waste products from the tumor site will only suffice for very small tumors. Blood vessels in the tumor's vicinity provide a route for detached tumor cells to enter the circulatory system and metastasize to secondary sites. Imbalances in angiogenic and lymphangiogenic processes are thus frequently involved in cancer and lend several biomarkers that indicate cancer progression and metastasis[9].

Vascular endothelial growth factor (VEGF) has been a key therapeutic target in the development of anti-angiogenic therapies to treat a range of cancers. Bevacizumab is a VEGF neutralizing antibody approved for use in a variety of cancer types, such as non-squamous non-small cell lung cancer and colorectal cancer, with potential in the treatment of epithelial ovarian cancer[10].

Lymphatic metastases are common, with a number of cancers first metastasizing to regional lymph nodes. Biomarkers for lymphangiogenesis and metastatic spread are being investigated, with VEGF-C and VEGF-D and VEGFR3 emerging as biomarkers for non-small cell lung cancer and breast cancer[11, 12].

Metastatic tumor development

Escaping circulation and establishing a metastatic tumor involves the integration of multiple factors and events, such as traversing the endothelial wall and transendothelial migration and angiogenesis. Consequently, many of the biomarkers of extravasation reflect earlier metastatic processes, such as VEGF, chemokines and cytokines, and ECM components, such as heparanase and matrix metalloproteinases[7, 6].

Additionally, the tumor microenvironment plays a key role in metastasis and offers a number of biomarkers for clinical utility.

References

1. Selves, J., Long-Mira, E., Mathieu, M., Rochaix, P., and Ilié, M. (2018). Immunohistochemistry for Diagnosis of Metastatic Carcinomas of Unknown Primary Site. *Cancers (Basel)*; **10**(4): 108.

2. Martin TA, Jiang WG. Loss of tight junction barrier function and its role in cancer metastasis. *Biochim Biophys Acta*. 2009; **1788**:872–91.

3. Bernardi MA, Logullo AF, Pasini FS, Nonogaki S, Blumke C, Soares FA, et al. Prognostic significance of CD24 and claudin-7 immunoexpression in ductal invasive breast cancer. *Oncol Rep*. 2012; **27**:28–38.

4. Chao YC, Pan SH,Yang SC,Yu SL, Che TF, Lin CW, et al. Claudin-1 is a metastasis suppressor and correlates with clinical outcome in lung adenocarcinoma. *Am J Respir Crit Care Med*. 2009; **179**:12333.

5. Richardson F,Young GD, Sennello R, Wolf J, Argast GM, Mercado P, et al. The evaluation of E-Cadherin and vimentin as biomarkers of clinical outcomes among patients with non-small cell lung cancer treated with erlotinib as second- or third-line therapy. *Anticancer Res.* 2012; **32**:537–52.

6. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduct Target Ther*. 2020 Mar **12**;5:28.

7. Martin TA,Ye L, Sanders AJ, et al. Cancer Invasion and Metastasis: Molecular and Cellular Perspective. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013.

8. Vergara D, Simeone P, Franck J, et al. Translating epithelial mesenchymal transition markers into the clinic: Novel insights from proteomics. *EuPA Open Proteom*. 2016; **10**:31-41.

9. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell*. 2011; **146**:87387.

10. Sato S, Itamochi H. Bevacizumab and ovarian cancer. *Curr Opin Obstet Gynecol.* 2012; **24**:8–13.

11. Al-Rawi MA, Jiang WG. Lymphangiogenesis and cancer metastasis. *Front Biosci*. 2011; **16**:723–39.

12. Albrecht I, Christofori G. Molecular mechanisms of lymphangiogenesis in development and cancer. *Int J Dev Biol*. 2011; **55**:483–94.

Validating your cancer biomarker antibody for IHC

Immunohistochemistry determines the cellular or subcellular localization of a specific protein. This technique plays a vital role in diagnostics and is increasingly used to target drug therapies as a quick and cost-effective assay[1,2]. Biomarkers for conditions such as cancer are commonly analyzed via IHC to yield essential diagnostic and prognostic information and to monitor treatments.

Interpreting positive and negative signals in IHC assays can be difficult. In addition to the acknowledged problems of antigen retrieval in paraffin-embedded sections, the differential availability of antigens between different assay formats means that an antibody recognizing a single band on a western blot may recognize multiple proteins in IHC.

There is no easy way around these problems. The usual approach is to compare samples to both positive and negative control sample staining and to demonstrate that similar signals can be obtained using multiple antibodies against the same target.

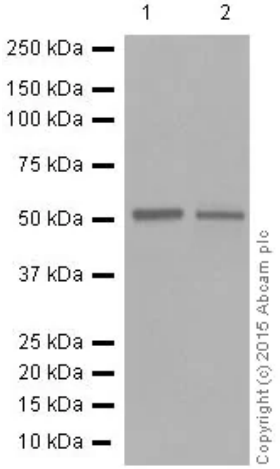
A crucial step prior to IHC analysis is antibody validation; this will ensure your antibody is specific to the cancer biomarker of interest and sufficiently sensitive to allow IHC analysis over the required dynamic range demanded by the pathology.

Early validation of your cancer biomarker antibody offers confidence in your results and allows a better understanding of the target. Work produced with validated antibodies ensures long-term reproducibility of results and is quicker to transition to the clinical setting[3].

At abcam, we perform rigorous antibody validation for IHC to guarantee antibody specificity, sensitivity and reproducibility. We include numerous reviews direct from researchers highlighting how our antibodies perform hands-on across many different labs.

Important IHC antibody validation steps

No cross-reactivity: Determine cross-reactivity and target specificity in an application such as western blotting[4]. This allows easy visualization of your antibody specificity.



Anti-ERG antibody [EPR3864] (ab92513) validation. Western blot analysis shows ERG at the expected mol weight of 55kDa with no cross-reactivity in Jurkat cells (lane 1) and HeLa cells (lane 2).

Correct IHC staining pattern: Use both positive and negative expressing tissue samples with known localization patterns to confirm the antibody still specifically and sensitively binds the target following formalin fixation and antigen retrieval processes.

Confirm testing in relevant tissue: Antibody testing in tissue samples that are representative of the end-use is important. This includes testing across healthy and related diseased tissues to confirm antibody specificity and sensitivity in your tissue of interest.

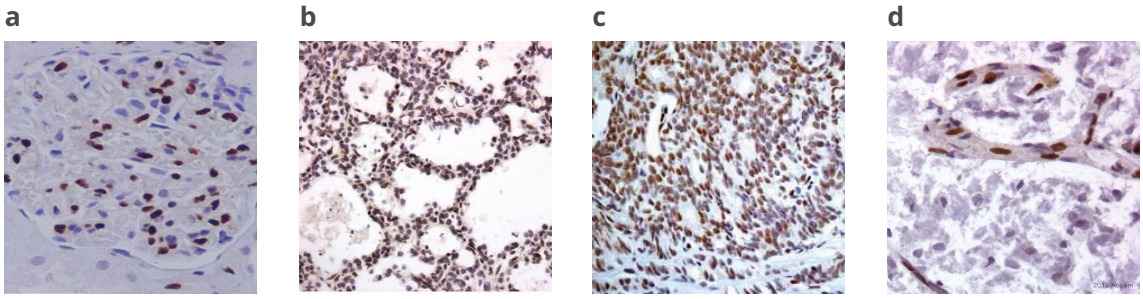


Figure: IHC staining of ERG in FFPE tissue samples. (a) Human kidney tissue with negative control inset (b) mouse brain tissue (c) human prostate cancer (d) human brain tissue with anti-ERG primary antibody (ab92513) show the expected protein localization in each tissue type.

The antibody is only considered specific if it repeatedly demonstrates the expected localization across all positive and negative controls.

Reproducible antibody performance: consistent antibody performance is critical to the long-term viability of research results. Recombinant antibodies are defined by their sequence, so can be reproducibly manufactured with the least batch-to-batch variation among affinity reagents[5].

Abcam recombinant antibodies offer excellent target specificity, sensitivity and reproducibility.

Validating reproducibility in IHC antibodies requires comparison for each new batch of antibody produced, to determine batch-to-batch consistency with reproducible, clear staining of the target protein in each of the relevant tissues.

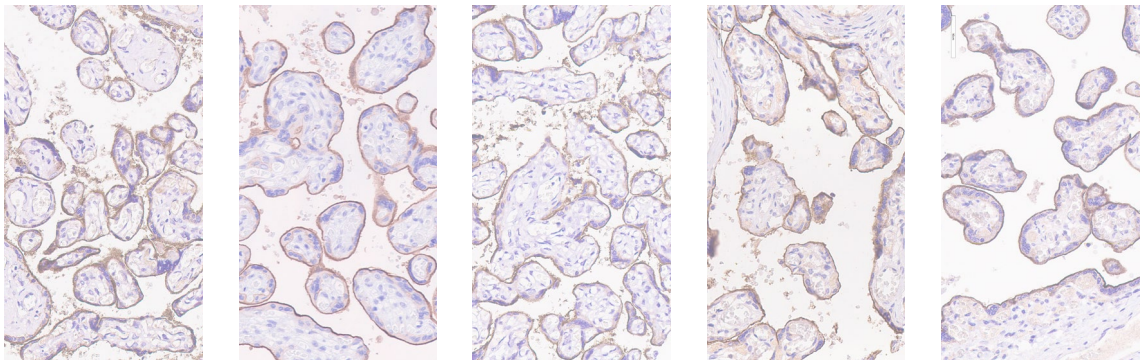


Figure: Batch-to-batch reproducibility in the localization of PD-L1 in the FFPE human placenta across five batches of Anti-PD-L1 antibody [28-8] (ab205921). IHC performed manually using a Biocare Decloaking Device and Universal HIER antigen retrieval reagent (ab208572). The primary antibody was incubated overnight at 4°C, followed by Goat anti-rabbit secondary antibody and HRP-linked anti-goat polymer antibody (ab209101). DAB was used as a chromogen with a hematoxylin counterstain.

Validating in your lab

We recommend that you perform initial in-house validation with your cancer biomarker antibody before you begin your experiments.

— For well-established IHC antibodies, your own in-house validation can be as straightforward as reproducing the expected result for your cancer biomarker antibody on positive and negative tissues to gain an appropriate signal[2].

— When investigating validated cancer biomarkers in a new tissue or sample preparation, or novel cancer biomarkers, your own validation may include positive control material from previously investigated tissue. Antibody staining and signal consistency should match those from previously validated tissues. This allows you to optimize your antibody for use in your sample of interest[2].

References

1. Yu J, Kane S, Wu J, et al. *Clin. Cancer Res.* 2009; **15**:3023–3028. 2.

2. Howat WJ, Lewis A, Jones P, et al. *Methods*. 2014; **70**(1): 34–38.

3. Deutsch EW, Ball CA, Berman JJ, et al. *Nat. Biotechnol*. 2008; **26**:305–312.

4. Schuster C, Malinowsky K, Liebmann S, et al. *Histopathology*. 2012; **60**:E37–E50. 5.

5. Bradbury, A. & Plückthun, A. *Nature* 2015; **518**, 27-29.

lung cancer biomarkers

1

Lung cancer biomarkers

Lung cancer is one of the leading causes of cancer-related death worldwide, accounting for approximately one-third of cancer-related deaths[1]. The diagnosis of different lung cancers relies heavily on the use of IHC-based diagnostic tests and continued research into lung cancer biomarkers that can be used in IHC.

There is an increasing number of antibodies available for detecting lung cancer-specific biomarkers used to determine different types of lung cancer and their cells of origin. These different cancer types include adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

Here we look at some of the most common primary IHC markers and metastatic markers for lung cancer. We also provide recommendations of specific biomarker antibodies for use in IHC.

Primary IHC lung cancer biomarkers

Aryl hydrocarbon receptor (AHR)

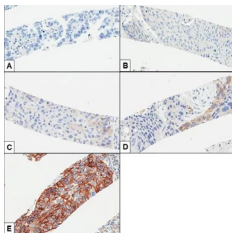
AHR is a transcription factor highly expressed in bronchial epithelial cells, where it affects cell proliferation, differentiation, and cell-cell adhesion. It plays a key role in diseases such as bronchitis and asthma, but it is also strongly implicated in the progression of lung cancer. It does this through the promotion of cell proliferation, angiogenesis, inflammation, and apoptosis[2]. AHR expression is also linked with smoking-related tumors as it may upregulate CYP1A1, releasing harmful factors into the lung tissue. It is, therefore, a marker of poor prognosis for lung cancer patients[2].

IHC stain (marker localization):
Nuclear and cytoplasm

View antibodies to AHR

Programmed death-1 ligand (PD-L1)

PD-L1 is a transmembrane protein that acts by inhibiting T cell activation and proliferation. PD-L1 protein detection by IHC testing is widely used as a predictive biomarker assay for anti–PD-1/PD-L1 therapies for several cancer types, including lung cancer. Non-small cell lung cancer (NSCLC) accounts for 75% of all lung cancers, and approximately 50% of NSCLC cases will have expression of PD-L1 in histology carried out on patient biopsies, making it a strong biomarker for this cancer type.



IHC stain (marker localization):
Cell membrane

Recommended IHC antibody:
Recombinant Anti-PD-L1 antibody [28-8] (ab205921)

View antibodies to PD-L1

Figure: Formalin-fixed, paraffin-embedded human lung cancer tissue stained for PD-L1 using ab205921. Reproduced under Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Nakamura et al PLoS One. 2017; 12(10): e0186192. Published online 2017 Oct 19. doi: 10.1371/journal.pone.0186192

Programmed death-1 (PD1) (PDCD1/CD279)

Programmed death-1 receptor is the receptor to transmembrane ligand PD-L1 (above) and is found to be expressed on the surface of T-cells. Tumors use this PD-1/ PD-L1 interaction to evade and suppress the immune response. These interactions are commonly seen in NSCLC as described above for PD-L1. Nivolumab, an anti-PD-1 drug, is approved for use in squamous NSCLC[3].

IHC stain (marker localization):
Cell membrane

Recommended IHC antibody:
Recombinant Anti-PD1 antibody [CAL20] (ab237728)

View antibodies to PD1

Surfactant protein A (SPA)

SPA is a large protein expressed within alveoli cells of the lung and is responsible for fighting infectious disease and reducing alveoli surface tension. SPA is also a biomarker used to detect adenocarcinoma in the lung. It is thought to be a marker of good prognosis as SPA is known to reduce tumor progression in the lung by recruiting natural killer cells to the site of the tumor[4].

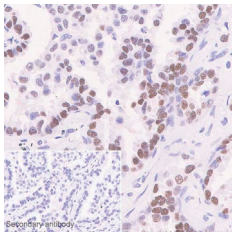
IHC stain (marker localization):
Secreted protein within the extracellular space

Recommended IHC antibody:
Recombinant Anti-ALK antibody [SP144] (ab183332)

View antibodies to SPA

SOX2

SOX2 is a transcription factor that plays a crucial role in the developing embryonic lung. It is also important for the formation of the proximal airways, where it is used as a marker of proliferation and lung stem cells. It is also found to be overexpressed in over 80% of patients with lung squamous cell carcinoma (LSCC), a type of NSCLC. It is therefore commonly used as a marker of lung cancer cells derived from this squamous cell lineage[5].



IHC stain (marker localization):
Nuclear

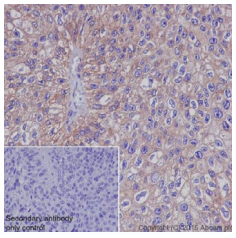
Recommended IHC antibody:
Recombinant Anti-SOX2 antibody [SP76] (ab93689)

View antibodies to SOX2

Figure: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue sections labeling SOX2 with ab93689 at 1:100. Negative control used PBS instead of primary antibody. Sections were counterstained with hematoxylin.

MET

The MET proto-oncogene is a transmembrane tyrosine kinase receptor, and its signaling cascade is involved in proliferation, apoptosis, and cellular migration. It also plays a role in the progression of NSCLC. Overexpression of MET in NSCLC leads to misregulation of proliferation and cell migration leading to a more aggressive cancer[6].



IHC stain (marker localization):
Cell membrane

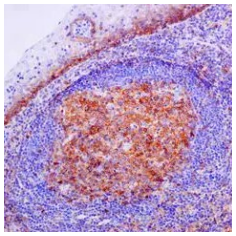
Recommended IHC antibody:
Recombinant Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067)

View antibodies to MET

Figure: Immunohistochemical staining of paraffin-embedded human bladder carcinoma with purified ab51067 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counterstained with hematoxylin.

Fas

Fas (or apoptosis antigen 1 APO-1) is a death receptor expressed on the cell surface which mediates apoptosis. Misregulation of apoptosis in normal cell types can lead to the progression of cancer. FasL (the FAS ligand) overexpression has been shown as a prognosis of advanced cancer stage in NSCLC[7].



IHC stain (marker localization):
Secreted protein and cell membrane

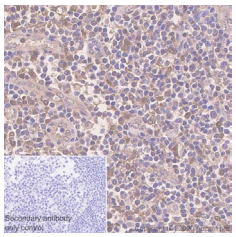
Recommended IHC antibody:
Recombinant Anti-Fas antibody [EPR5700] (ab133619)

View antibodies to Fas

Figure: Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD95 with ab133619 antibody at a dilution of 1/250.

Glutathione S-transferase pi 1 (GSTP1)

GSTP1 is an enzyme responsible for breaking down toxic compounds. In the lung, GSTP1 is highly expressed and is known to carry out the metabolism of carcinogens carried into the lung through smoking. Certain variants of GSTP1 are used as markers of lung cancer prognosis. The Ile105Val variant is associated with a reduced risk of lung cancer and a reduction in mortality from the disease[8].



IHC stain (marker localization):
Cytoplasm

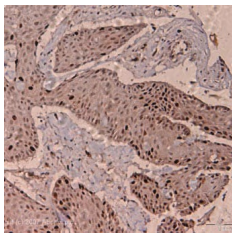
Recommended IHC antibody:
Recombinant Anti-GST3 / GST pi antibody [EPR8263] (ab138491)

View antibodies to GSTP1

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling GST3 / GST pi with Purified ab138491 at 1/100 dilution (0.99 µg/mL).

Ki67

Ki67 is an essential protein involved in cell division and is commonly used as a marker of cellular proliferation. IHC staining for Ki-67 is a commonly used method for evaluating proliferative activity in various tumor types, including lung tumors. Studies suggest a key role of Ki-67 as a prognostic marker of NSCLC[9].



IHC stain (marker localization):
Nuclear

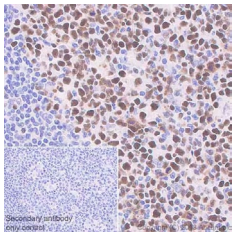
Recommended IHC antibody:
Recombinant Anti-Ki67 antibody [SP6] (ab16667)

View antibodies to Ki67

Figure: IHC image of ab15580 stained human skin carcinoma FFPE section. Section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH=6) for 30 seconds at 125°C. Section was incubated with ab15580 at a dilution of 1:200 for 1h at room temperature and detected using an HRP conjugated polymer system.

MCM7

MCM7 is a chromosomal maintenance protein important during the cell cycle and has been associated with various cancer types, including lung cancers. Both MCM7 and ki67 (the proliferation marker) are highly expressed in squamous cell carcinomas of the lung. Both are associated with poor prognosis in the disease. MCM7 can also be used as an IHC biomarker from bronchial brushings[10].



IHC stain (marker localization):
Nuclear

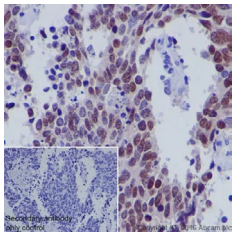
Recommended IHC antibody:
Recombinant Anti-MCM7/PRL antibody [EP1974Y] (ab52489

View antibodies to MCM7

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labeling MCM7/PRL with purified ab52489 at 1/50 dilution (5.4 µg/mL). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

Achaete-scute complex 1 (ASCL1)

ASCL1 is a transcription factor necessary for neuroendocrine lung development and the growth of both SCLC and NSCLC. It acts as a marker of poor prognosis in NSCLC and looks promising as a potential druggable target for the treatment of NSCLC[11].



IHC stain (marker localization):
Mitochondrion outer membrane; Peroxisome membrane; Microsome membrane; Endoplasmic reticulum membrane

Recommended IHC antibody:
Recombinant Anti-MASH1/Achaete-scute homolog 1 antibody [EPR19592] (ab213151)

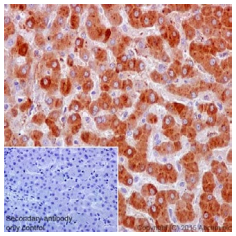
View antibodies to ASCL1

Figure: Immunohistochemical analysis of paraffin-embedded human lung small cell carcinoma tissue labeling MASH1/Achaete-scute homolog 1 with ab213151 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) at 1/500 dilution.

Metastatic markers

C-reactive protein (CRP)

Increased levels of CRP are an indication of inflammation. It is commonly seen to be expressed in lung cancer patients who smoke. Smoking leads to chronic lung inflammation and the upregulation of several inflammation response genes, including CRP. Expression of CRP is a marker of lung squamous cell carcinomas and small-cell cancers but not adenocarcinoma of the lung[12].



IHC stain (marker localization):
Secreted protein

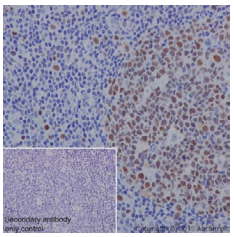
Recommended IHC antibody:
Recombinant Anti-C Reactive Protein antibody [Y284] (ab32412)

View antibodies to CRP

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labeling C Reactive Protein with purified ab32412 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500).

MCM6

MCM6 is a chromosomal maintenance protein important for the cell cycle and mitosis and has been associated with increasing the metastatic potential of various cancer types, including lung cancers. It has been shown to be expressed in around 50% of NSCLC patient samples, and high expression levels of MCM6 is linked to a poor prognosis of this disease. Its expression is also seen to be higher in patients who smoke[13].



IHC stain (marker localization):
Nuclear

Recommended IHC antibody:
Anti-MCM6 antibody [EPR17686] (ab201683)

View antibodies to MCM6

Figure: Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling MCM6 with ab201683 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

References

1. Siegel R, Naishadham D and Jemal A: Cancer statistics, CA *Cancer J Clin* **62**: 10-29 (2012).

2. Tsay, J. J., et al,. Aryl hydrocarbon receptor and lung cancer. *Anticancer Research*, **33**(4), 1247–1256 (2013).

3. Chen, Y., Mu, C., Huang, J. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. *Tumori* **98**: 751–755 (2012).

4. Mitsuhashi, A., et al, Surfactant protein a suppresses lung cancer progression by regulating the polarization of tumor-associated macrophages *American Journal of Pathology*, **182**(5), 1843–1853 (2013).

5. Mollaoglu, G., et al., The lineage defining transcription factors SOX2 and NKX2-1 determine lung cancer cell fate and shape the tumor immune microenvironment. *Immunity*, **49**(4), 764–779 (2019).

6. Salgia, R. MET in lung cancer: Biomarker selection based on scientific rationale. *Molecular Cancer Therapeutics*, **16**(4), 555–565 (2017).

7. Viard-Leveugle, I., et al, Frequent loss of Fas expression and function in human lung tumours with overexpression of FasL in small cell lung carcinoma. *Journal of Pathology*, **201**(2), 268–277 (2003).

8. Nørskov, M. S., Dahl, M., and Tybjærg-Hansen, A. Genetic Variation in GSTP1, Lung Function, Risk of Lung Cancer, and Mortality. *Journal of Thoracic Oncology*, **12**(11), 1664–1672 (2017).

9. Kriegsmann, M., & Warth, A. Ki-67 expression in pulmonary tumors-reply. *Translational Lung Cancer Research*, **5**(5), 552–553 (2016).

10. Liu, Y. Z., et al., Prognostic significance of MCM7 expression in the bronchial brushings of patients with non-small cell lung cancer (NSCLC). *Lung Cancer*, **77**(1), 176–182 (2012).

11. Augustyn, A., et al., ASCL1 is a lineage oncogene providing therapeutic targets for high-grade neuroendocrine lung cancers. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(41), 14788–14793 (2014).

12. Chaturvedi, A. K., et al., C-reactive protein and risk of lung cancer. *Journal of Clinical Oncology*, **28**(16), 2719–2726 (2010).

13. Liu, Y. Z., et al., MCMs expression in lung cancer: Implication of prognostic significance. *Journal of Cancer*, **8**(18), 3641–3647 (2017).

breast cancer biomarkers

2

Breast cancer biomarkers

Emerging breast cancer biomarkers

ALDH1A1

Aldehyde dehydrogenase 1 family member A1 (ALDH1A1) has been identified as a putative cancer stem cell marker in breast cancer[1].

View antibodies to ALDH1A1

SLFN11

Schlafen 11 (SLFN11) is emerging as an important regulator of cellular response to DNA damage. Preclinical and emerging clinical trial data suggest that SLFN11 is a predictive biomarker of response to a wide range of therapeutics that cause DNA damage, raising exciting possibilities for its clinical application[2].

View antibodies to SLFN11

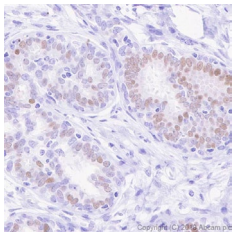
IHC is a common method used in the diagnosis of breast cancer and research into breast cancer pathology. These markers are used to determine different breast cancer types, e.g. *in situ* or invasive carcinoma, distinguishing normal breast cell types, e.g. luminal, basal and myoepithelial, and also proliferation and disease progression[3].

Here we look at some of the most common primary IHC markers for breast cancer and some cell type specific biomarkers. We also recommend specific IHC antibodies for each biomarker.

Established breast cancer biomarkers

Estrogen receptor alpha

Determining the distribution of estrogen receptor alpha (ER-α) in breast cancer samples is an important initial step for the diagnosis and treatment evaluation of the disease[4]. Approximately 70% of breast cancer samples will give a positive staining signal for ER-α, making it a crucial biomarker for breast cancer diagnosis[5]. ER-α is a nuclear protein with a ligand-dependent transcription factor function. It is also most commonly detected in both luminal A and B subtypes of breast cancer[5].



IHC stain (marker localization):
Nuclear stain

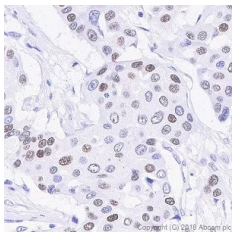
Recommended IHC antibody:
Recombinant Anti-Estrogen Receptor alpha antibody [E115] - ChIP Grade (ab32063)

View antibodies to Estrogen receptor alpha

Figure: Immunohistochemical staining of paraffin-embedded human breast carcinoma tissue with ab32063 at a dilution of 1/5000. The secondary antibody used was Goat Anti-Rabbit IgG H&L (HRP Polymer). The sample is counter-stained with hematoxylin. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0).

Progesterone receptor

The progesterone receptor (PR) is another biomarker important for the initial diagnosis and evaluation of breast cancer[4]. PR is known to be induced by ER-α and plays a key role in ER-α protein regulation. PR as a biomarker is therefore commonly used as an indicator of ER-α function[6] . PR is highly expressed in luminal A-type breast cancer tissue and is associated with a good prognosis[7].



IHC stain (marker localization):
Nuclear

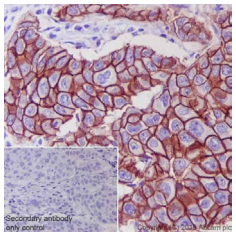
Recommended IHC antibody:
Recombinant Anti-Progesterone Receptor antibody [SP42] (ab101688)

View antibodies to Progesterone receptor

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling progesterone receptor with ab101688 at 1/400 dilution. Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Hematoxylin was used as a counterstain. Nuclear staining on human breast cancer tissue, performed on a Leica Biosystems BOND® RX instrument.

ErbB2/HER2

Overexpression of ErbB2/HER2 can be found in 20–30% of breast cancer tumors and is commonly found in more aggressive types of the disease[8]. ErbB2/HER2 is used as a diagnostic indicator for the FDA approved monoclonal antibody therapy, Trastuzumab (Herceptin). During this therapy ErbB2/HER2 acts as the binding target of Trastuzumab[9].



IHC stain (marker localization):
Cytoplasmic, nuclear, and strongly in the cell membrane

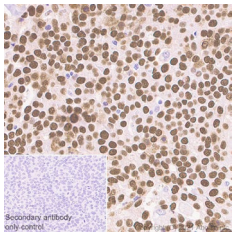
Recommended IHC antibody:
Recombinant Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

View antibodies to HER2

Figure: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling ErbB2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

p53

Mutated versions of the tumor suppressor p53 can be found in 80% of triple-negative breast cancer (TNBC) cases, ie tumors lacking expression of ER, PR, and ErbB2/HER2[10]. This makes mutant p53 an excellent biomarker for TNBC, one of the more difficult breast cancers to treat as it will not respond to endocrine or anti-HER2 treatments[11]. Currently, there are mixed findings on the prognostic potential of p53 expression in breast cancer. Some studies have associated it with a positive prognostic outcome and others – with negative[11]. p53 IHC stains are commonly used for diagnostic purposes.



IHC stain (marker localization):
Cytoplasm and strong nuclear

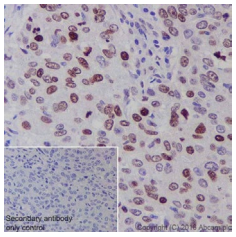
Recommended IHC antibody:
Recombinant Anti-p53 antibody [E26] (ab32389)

View antibodies to p53

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium cancer tissue sections labeling p53 with purified ab32389 at 1/5000 dilution (0.09 µg/mL).

BRCA1

Mutations in the BRCA genes are some of the most well-known breast cancer-associated protein mutations. BRCA1 is a tumor suppressor gene and one of the most commonly mutated genes in breast cancer. Patients with a BRCA1 mutation have a 40–80% increased risk of developing the disease, making it a crucial biomarker in the diagnosis and prognosis of many breast cancer types[12].



IHC stain (marker localization):
Nuclear signal

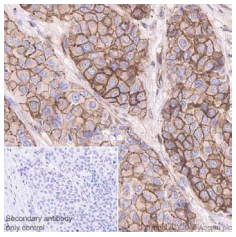
Recommended IHC antibody:
Recombinant Anti-BRCA1 antibody [EPR19433] (ab213929)

View antibodies to BRCA1

Figure: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling BRCA1 with ab213929 at 1/400 dilution, followed by rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Epidermal growth factor receptor (EGFR)

EGFR is a transmembrane receptor, and its expression is frequently found in TNBC and inflammatory breast cancer (IBC). It is most commonly associated with a poor prognosis[13]. High levels of EGFR expression is linked to an increased ability of breast cancer to undergo metastasis, making it a common biomarker for aggressive breast cancer types[14].



IHC stain (marker localization):
Cytoplasm and strong nuclear

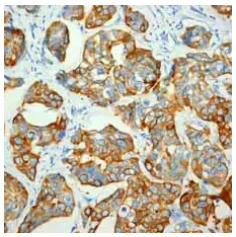
Recommended IHC antibody:
Recombinant Anti-EGFR (phospho Y1068) antibody [EP774Y] (ab40815)

View antibodies to EGFR

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling EGFR with purified ab40815 at 1:500 dilution (1.75 µg/mL). Heat-mediated antigen retrieval was performed using heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).

Cytokeratin 7 (CK7) and Cytokeratin 20 (CK20)

CK7 and CK20 are cytokeratins expressed in breast epithelia. Different CK7 and CK20 IHC expression patterns are commonly used to distinguish many carcinoma types, including breast carcinomas. Most breast cancers are CK7 positive and CK20 negative, making the combination of these cytokeratins an excellent biomarker combination. Approximately 80% of breast adenocarcinomas are CK7 positive and CK20 negative[15].

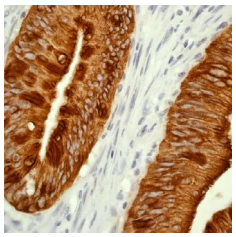


IHC stain (marker localization):
Cytoplasm

Recommended CK7 IHC antibody:
Recombinant Anti-Cytokeratin 7 antibody [EPR1619Y] - Cytoskeleton Marker (ab68459)

View antibodies to CK7

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labeling Cytokeratin 7 with unpurified ab68459.



IHC stain (marker localization):
Cytoplasm

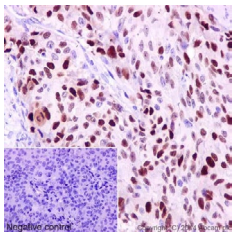
Recommended CK20 IHC antibody:
Recombinant Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126)

View antibodies to CK20

Figure: ab76126 at 1/100 dilution staining Cytokeratin 20 in human colon adenocarcinoma by immunohistochemistry, paraffin-embedded tissue.

Cyclin D1

Cyclin D1 is one of the main regulatory proteins of the cell cycle, promoting the cell cycle progression from G1 to S phase. Mutations in cyclin D1 are amplified in many different types of cancer, including many breast cancers. 50% of breast cancers will contain an overexpression of cyclin D1[16]. It has been used as a biomarker for poor prognosis for many breast cancer types, but some studies are beginning to suggest that it could also indicate a positive outcome if expressed in some luminal subtypes of breast cancer[17].



IHC stain (marker localization):
Nuclear

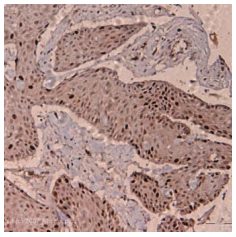
Recommended IHC antibody:
IHC antibody: Recombinant Anti-Cyclin D1 antibody [EPR2241] - C-terminal (ab134175)

View antibodies to Cyclin D1

Figure: Immunohistochemical staining of paraffin-embedded human endometrial adenocarcinoma with purified ab134175 at a dilution of 1/100. An HRP goat anti-rabbit (ab97051) was used as the secondary antibody at a dilution of 1/500 and the sample was counterstained with hematoxylin.

Ki67

Ki67 is an important protein involved in cell division and is commonly used as a marker of cellular proliferation. It is also used in breast cancer diagnosis to determine the level of cell proliferation, which can be a prognostic marker and a good indication of how well certain breast cancers will respond to endocrine therapy[18].



IHC stain (marker localization):
Nuclear

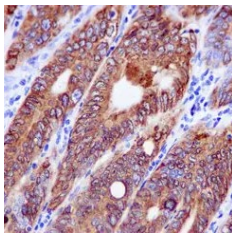
Recommended IHC antibody:
Recombinant Anti-Ki67 antibody [SP6] (ab16667)

View antibodies to Ki67

Figure: IHC image of ab15580 stained human skin carcinoma FFPE section. Section was pre-treated using pressure cooker heat-mediated antigen retrieval with sodium citrate buffer (pH=6) for 30 seconds at 125°C. Section was incubated with ab15580 at a dilution of 1:200 for 1h at room temperature and detected using an HRP conjugated polymer system.

Cyclooxygenase-2 (COX-2)

COX-2 is an enzyme responsible for producing prostanoids. Its expression in breast tissue correlates strongly with breast cancer development. Overexpression of COX-2 has been shown to drive breast cancer phenotypes, and blocking the action of this protein also shows potential for breast cancer therapy. There is also a strong link between COX-2 overexpression in breast adipose tissue and cancer progression linked to obesity[19].



IHC stain (marker localization):
Cytoplasm

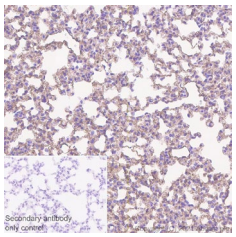
Recommended IHC antibody:
Recombinant Anti-COX2 / Cyclooxygenase 2 antibody [EPR12012] (ab179800)

View antibodies to COX2

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic carcinoma tissue labeling COX2/Cyclooxygenase 2 with unpurified ab179800 at a dilution of 1/250.

Caveolin-1

Caveolin-1 is the main component of caveolae, small invaginations found in the cell membrane. It's also known to play a key role in cell proliferation, invasion, and breast cancer metastasis. It is also used as a biomarker for breast cancer treatments and disease outcome. High expression of Caveolin-1 is a sign of poor prognosis and indicates more aggressive metastatic breast cancer[20].



IHC stain (marker localization):
Cell membrane

Recommended IHC antibody:
Recombinant Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

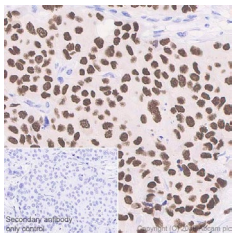
View antibodies to Caveolin-1

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse lung tissue sections labeling Caveolin-1 with purified ab32577 at 1/2000 dilution (0.06 µg/mL). Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) secondary antibody was used at 1:0 dilution.

Cell type specific biomarkers

FOXA1

The transcription factor FOXA1 has been shown to have a unique distribution within breast cancer cells compared to other cell types. FOXA1 binding is crucial for chromatin opening and the transcriptional activation of ER-α responsive genes within breast cancer cells[21]. Studies have identified thousands of breast cancer-associated single nucleotide polymorphisms (SNPs) within the enhancer regions for FOXA1[22].



IHC stain (marker localization):
Nuclear signal

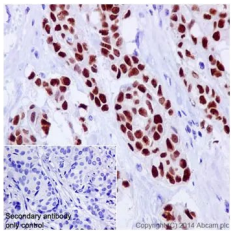
Recommended IHC antibody:
Recombinant Anti-FOXA1 antibody [EPR10881] (ab170933)

View antibodies to FOXA1

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling FOXA1 with purified ab170933 at 1:1000 dilution (1.13 µg/mL). Heat-mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).

GATA-binding protein 3 (GATA3)

GATA3 is a zinc finger transcription factor crucial for breast luminal epithelium differentiation. It is also a diagnostic biomarker for both primary and metastatic breast cancer and commonly found in luminal A and B breast cancer tissue. GATA3 also has a strong association with HER2-positive and ER-α positive hormone response in luminal breast cancers[23].



IHC stain (marker localization):
Nuclear signal

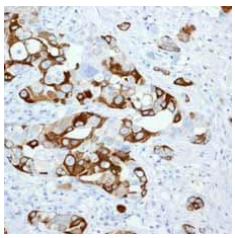
Recommended IHC antibody:
Recombinant Anti-GATA3 antibody [EPR16651] - ChIP Grade (ab199428)

View antibodies to GATA3

Figure: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling GATA3 with ab199428 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human breast carcinoma tissue is observed. Counterstained with hematoxylin.

Cytokeratin 5 (CK5)

CK5 is a cytokine found to be expressed in the basal cell layer of the mammary duct. Tumors that arise from these cells are also known to be CK5 positive, so CK5 is an excellent biomarker for the diagnosis of basal type breast cancers. The CK5-positive progenitor cells may also differentiate into glandular and myoepithelial cancer types[24].



IHC stain (marker localization):
Cytoplasm and cell membrane

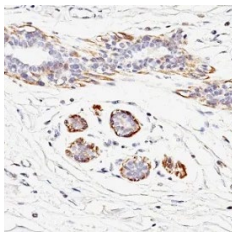
Recommended IHC antibody:
Recombinant Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

View antibodies to CK5

Figure: Unpurified ab52635 showing positive staining in basal cell breast carcinoma tissue.

Smooth muscle myosin heavy chain (SMMHC)

SMMHC is expressed specifically in the contractile myoepithelial cells of the breast. A loss of the myoepithelial layer is commonly associated with invasive breast cancers. Markers such as SMMHC can be used in IHC to detect this layer more reliably than a hematoxylin and eosin stain alone[3].



IHC stain (marker localization):
Cytoplasm

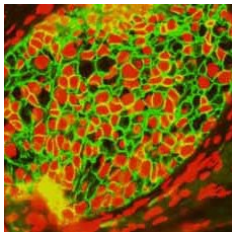
Recommended IHC antibody:
Anti-smooth muscle Myosin heavy chain I antibody [MYH11/923] (ab233940)

View antibodies to SMMHC

Figure: Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for smooth muscle Myosin heavy chain I using ab233940 at 1 µg/mL in immunohistochemical analysis.

E-cadherin

E-cadherin is expressed at the cell junction of normal breast epithelial cells. A loss of E-cadherin is associated with a phenotypic switch by these cells to become invasive and migratory breast cancer cells. Loss of E-cadherin expression is commonly used as a biomarker for metastatic lobular breast carcinomas[25].



IHC stain (marker localization):
Cell junctions

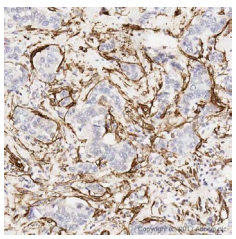
Recommended IHC antibody:
Recombinant Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772)

View antibodies to E-cadherin

Figure: Fluorescent immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab40772. Green-E-Cadherin red-PI.

Alpha smooth muscle actin (ACTA2)

Smooth muscle actin is highly expressed in normal breast myoepithelial cells. It is commonly used as a marker of this normal breast cell type. Smooth muscle actin is commonly used for histology in conjunction with SMMHC and calponin to mark out the myoepithelial layer. A combination of markers such as this is much more reliable than using hematoxylin and eosin stains alone[26].



IHC stain (marker localization):
Cytoplasm

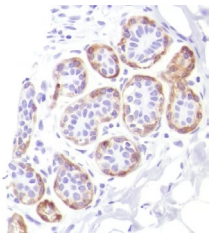
Recommended IHC antibody:
Recombinant Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

View antibodies to ACTA2

Figure: IHC image of alpha smooth muscle actin staining in a human breast ductal carcinoma formalin-fixed paraffin-embedded tissue section, performed on a Leica Bond™ system using the standard protocol F.

Calponin

Calponins are proteins found within the contractile components of the myoepithelium. Similarly to SMMHC and ACTA2, calponin stains are used as a marker of the myoepithelial layer in the diagnosis of invasive breast cancers[3].



IHC stain (marker localization):
Cytoplasm

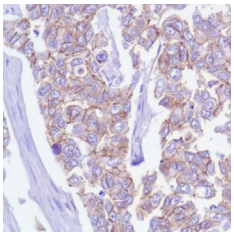
Recommended IHC antibody:
Recombinant Anti-Calponin 1 antibody [SP181] (ab227661)

View antibodies to Calponin

Figure: Formalin-fixed, paraffin-embedded human breast tissue stained for Calponin 1 using ab227661 at 1/100 dilution in immunohistochemical analysis.

Delta-1-Catenin

Delta-1-Catenin is a member of the p120 family and acts as a binder of E-cadherin. Delta-1-Catenin can be used in histology to detect invasive lobular breast cancer, high tumor-node-metastasis stage, and lymph node metastasis. It is also strongly associated with HER2-positive breast cancers and can be used as an indicator of poor prognosis as it is thought to promote a malignant phenotype[26].



IHC stain (marker localization):
Cytoplasm and cell membrane

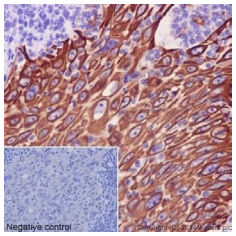
Recommended IHC antibody:
Recombinant Anti-delta 1 Catenin/CAS antibody [SP63] - C-terminal (ab227638)

View antibodies to Delta-1-Catenin

Figure: Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for delta 1 Catenin/CAS using ab227638 at 1/100 dilution in immunohistochemical analysis.

Cytokeratin 14 (CK14)

CK14 is a cytokine found to be expressed in the basal cell layer of the mammary duct. It is most commonly found to be co-expressing with CK5 in this tissue type. And similarly to CK5, the histological detection of CK14 is an excellent biomarker for the diagnosis of basal type breast cancers[24].



IHC stain (marker localization):
Cytoplasm

Recommended IHC antibody:
Recombinant Anti-Cytokeratin 14 antibody [EPR17350] - Cytoskeleton Marker (ab181595)

View antibodies to CK14

Figure: Immunohistochemical analysis of paraffin-embedded human squamous cell carcinoma of cervix tissue labeling Cytokeratin 14 with ab181595 at 1/2000 dilution, followed by prediluted HRP polymer for Rabbit/Mouse IgG.

References

1. Liu, Y., Lv, Dl., Duan, Jj. et al. ALDH1A1 expression correlates with clinicopathologic features and poor prognosis of breast cancer patients: a systematic review and meta-analysis. *BMC Cancer* **14**, 444 (2014).

2. Zhang, B., Ramkumar, K., Cardnell, R.J. et al. A wake-up call for cancer DNA damage: the role of Schlafen 11 (SLFN11) across multiple cancers. *Br J Cancer* **125**, 1333–1340 (2021).

3. Zaha, D C (2014) Significance of immunohistochemistry in breast cancer *World Journal of Clinical Oncology*, **5**(3), 382–392.

4. Hicks, D., Dell'Orto, P., Falzon, M., Hoff, K. D., Levy,Y.Y., McMahon, L., ... Viale, G. (2017). Immunohistochemical performance of estrogen and progesterone receptor antibodies on the dako omnis staining platform: Evaluation in multicenter studies. *Applied Immunohistochemistry and Molecular Morphology*, **25**(5), 313–319.

5. Jeselsohn, R., Buchwalter, G., De Angelis, C., Brown, M., & Schiff, R. (2015). ESR1 mutations-a mechanism for acquired endocrine resistance in breast cancer. *Nature Reviews Clinical Oncology*, **12**(10), 573–583.

6. Mohammed, H., Russell, I. A., Stark, R., Rueda, O. M., Hickey, T. E., Tarulli, G.A., ... Carroll, J. S. (2015). Progesterone receptor modulates ERα action in breast cancer. *Nature*, **523**(7560), 313–317.

7. Lim, E., Palmieri, C., & Tilley, W. D. (2016). Renewed interest in the progesterone receptor in breast cancer. *British Journal of Cancer*, **115**(8), 909–911.

8. Mitri, Z., Constantine,T., & O'Regan, R. (2012). The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. *Chemotherapy Research and Practice*, 2012, 1–7.

9. Piccart-Gebhart M.J... and Richard D. Gelber, Ph.D., for the Herceptin Adjuvant (HERA) Trial Study Team (2005). Trastuzumab after Adjuvant Chemotherapy in HER2-Positive Breast Cancer. *New England Journal of Medicine*, **353**;16.

10. Duffy, M. J., Synnott, N. C., & Crown, J. (2018). Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. *Breast Cancer Research and Treatment*, **170**(2), 213–219.

11. Li, J. P., Zhang, X. M., Zhang, Z., Zheng, L. H., Jindal, S., & Liu,Y. J. (2019). Association of p53 expression with poor prognosis in patients with triple-negative breast invasive ductal carcinoma. *Medicine*, **98**(18), e15449.

12. Fackenthal, J. D., & Olopade, O. I. (2007). Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nature Reviews Cancer*, **7**(12), 937–948.

13. Masuda, H., Zhang, D., Bartholomeusz, C., Doihara, H., Hortobagyi, G. N., & Ueno, N. T. (2012). Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Research and Treatment*, **136**(2), 331–345.

14. Ali, R., & Wendt, M. K. (2017).The paradoxical functions of EGFR during breast cancer progression. *Signal Transduction and Targeted Therapy*, **2** (2016), 1–7.

15. Chu, P., Wu, E., & Weiss, L. M. (2000). Cytokeratin 7 and Cytokeratin 20 expression in epithelial neoplasms: A survey of 435 cases. *Modern Pathology*, **13**(9), 962–972.

16. Mohammadizadeh. F., Hani M., Ranaee M., and Bagheri M. (2013). Role of cyclin D1 in breast carcinoma. *J Res Med Sci.* **18**(12) 1021–1025.

17. Ortiz, A. B., Garcia, D., Vicente, Y., Palka, M., Bellas, C., & Martin, P. (2017). Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. *PLoS ONE*, **12**(11), 1–13.

18. Niazi, M. K. K., Senaras, C., Pennell, M., Arole, V., Tozbikian, G., & Gurcan, M. N. (2018). Relationship between the Ki67 index and its area based approximation in breast cancer. *BMC Cancer*, **18**(1), 1–9.

19. Harris, R. E., Casto, B. C., & Harris, Z. M. (2014). Cyclooxygenase-2 and the inflammogenesis of breast cancer. *World Journal of Clinical Oncology*, **5**(4), 677–692.

20. Qian, X. L., Pan, Y. H., Huang, Q. Y., Shi, Y. B., Huang, Q.Y., Hu, Z. Z., & Xiong, L. X. (2019). Caveolin-1: A multifaceted driver of breast cancer progression and its application in clinical treatment. *OncoTargets and Therapy*, **12**, 1539–1552.

21. Cowper-Sallari, R., Zhang, X., Wright, J.B., Bailey, S. D., M. D., Eeckhoute, J., Moore, J.H., and L, Mathieu (2012). Breast cancer risk-associated SNPs modulate the affinity of chromatin for FOXA1 and alter gene expression. *Nat Genet*, **44**(11): 1191–1198.

22. Meyer, K B , & Carroll, J S (2012) FOXA1 and breast cancer risk *Nature Genetics*, **44**(11), 1176–1177.

23. Shaoxian, T., Baohua, Y., Xiaoli, X., Yufan, C., Xiaoyu, T., Hongfen, L., ... Wentao, Y. (2017). Characterisation of GATA3 expression in invasive breast cancer: Differences in histological subtypes and immunohistochemically defined molecular subtypes. *Journal of Clinical Pathology*, **70**(11), 926–934.

24. Laakso, M., Loman, N., Borg, Å., & Isola, J. (2005). Cytokeratin 5/14-positive breast cancer: True basal phenotype confined to BRCA1 tumors. *Modern Pathology*, **18**(10), 1321–1328.

25. Singhai, R., Patil, V. W., Jaiswal, S. R., Patil, S. D., Tayade, M. B., & Patil, A. V. (2011). E-Cadherin as a diagnostic biomarker in breast cancer North American *Journal of Medical Sciences*, **3**(5), 227–233.

26. Zhang, D., Zhang, J. Y., & Wang, E. H. (2015). Δ-Catenin promotes the malignant phenotype in breast cancer. *Tumor Biology*, **36**(2), 569–575.

colorectal cancer biomarkers

3

Colorectal cancer biomarkers

Emerging colorectal cancer biomarkers

NOTUM

NOTUM is a key mediator during the early stages of mutation fixation that can be targeted to restore wild-type cell competitiveness and provide preventative strategies for people at a high risk of developing colorectal cancer[1].

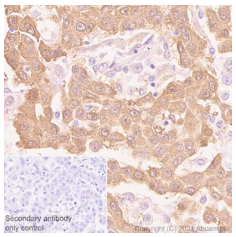
View antibodies to NOTUM

Here we look at some of the most common primary IHC markers for colorectal cancer (CRC) and cell type specific biomarkers. We also recommend specific antibodies for each biomarker for use in key applications such as IHC.

Established colorectal cancer biomarkers

Calretinin

Calretinin is a calcium-binding protein that plays a role in message targeting and intracellular calcium buffering. While absent in normal colonocytes, it is expressed in most poorly differentiated colon carcinomas[2]. Strongly positive calretinin staining in colon medullary carcinoma, as well as correlations between the expression of calretinin and degree of differentiation in human colorectal adenocarcinomas, have been reported emphasizing the potential of calretinin as an IHC biomarker for bowel cancer[3,4].



IHC stain (marker localization):
Cytoplasmic and nuclear

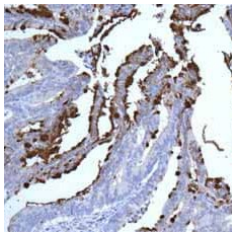
Recommended IHC antibody:
Recombinant Anti-Calretinin antibody [EP1798] (ab92341)

View antibodies to Calretinin

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human mesothelioma tissue sections labeling Calretinin with purified ab92341 at 1:4000 (0.034 µg/mL).

MUC2

MUC2 is part of the mucin family and characteristically observed in goblet cells of small and large bowel mucosa[5]. It is involved with coating the epithelia of the intestines, airways, and other mucus membrane-containing organs. Loss of MUC2 expression has been reported to be a predictor of adverse outcomes, and further prospective studies that evaluate adjuvant chemotherapy in stages II and III colon cancer should include MUC2 expression analysis for patient stratification[5].



IHC stain (marker localization):
Intestine, secreted into the inner and outer mucus layers

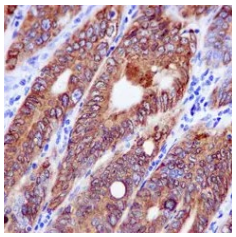
Recommended IHC antibody:
Recombinant Anti-MUC2 antibody [EPR6145] (ab134119)

View antibodies to MUC2

Figure: Immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using unpurified ab134119 showing +ve staining.

COX-2

COX-2 is an inducible enzyme that regulates prostaglandin synthesis and is overexpressed in several epithelial cancers. It is involved in the regulation of apoptosis, angiogenesis, and tumor cell invasiveness, which contribute to its effects on tumorigenesis. Multiple studies have shown that selective COX-2 inhibitors are a great targeted approach to the chemoprevention of CRC[6,7].



IHC stain (marker localization):
Microsome membrane and endoplasmic reticulum membrane

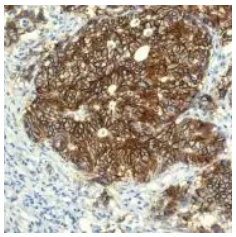
Recommended IHC antibody:
Recombinant Anti-COX2/Cyclooxygenase 2 antibody [EPR12012] (ab179800)

View antibodies to COX-2

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic carcinoma tissue labeling COX2/Cyclooxygenase 2 with unpurified ab179800 at a dilution of 1/250.

LI Cadherin

LI Cadherin, is one of a group of cadherins that act as calcium-dependent cell adhesion proteins that preferentially interact with themselves in a homophilic manner in connecting cells[8]. High LI Cadherin expression has been associated with liver metastasis, a major cause of death associated to colorectal cancer, and poor survival of patients[9].



IHC stain (marker localization):
Cell membrane

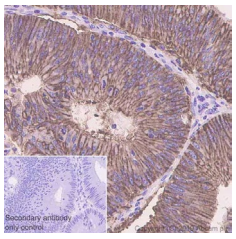
Recommended IHC antibody:
Recombinant Anti-LI Cadherin antibody [EPR3996] (ab109190)

View antibodies to LI Cadherin

Figure: Immunohistochemical staining of paraffin-embedded human colonic adenocarcinoma tissue using ab109190 at a dilution of 1/250.

GPA33

The GPA33 gene codes for A33 antigen, a transmembrane glycoprotein that is expressed in normal colonic and small bowel epithelium as well as more than 95% of human colon cancers[10]. IHC in CRC tissue has shown strong A33 membrane staining in samples of well-differentiated tumors[11], and there are also proposals for using antibodies to GPA33 as a potentially potent radioimmunotherapy regimen for GPA-33 positive CRC tumors in humans[12].



IHC stain (marker localization):
Cell membrane

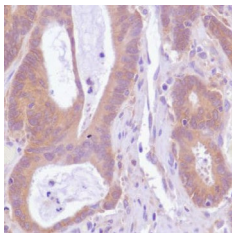
Recommended IHC antibody:
Recombinant Anti-GPA33 antibody [EPR4240] (ab108938)

View antibodies to GPA33

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon cancer tissue sections labeling GPA33 with purified ab108938 at 1:400 dilution (0.25 µg/mL). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

EGFR

Epidermal growth factor receptor (EGFR) is usually involved in cell growth and is recognized as an important player in CRC initiation and progression[13]. In late-stage colorectal cancer, the most commonly used targeted therapies are monoclonal antibodies that prevent EGFR activation[14]. However, future development of anti-EGFR directed nanoparticles that could inhibit overactive EGFR signals could potentially reduce CRC risk[15].



IHC stain (marker localization):
Secreted and cell membrane

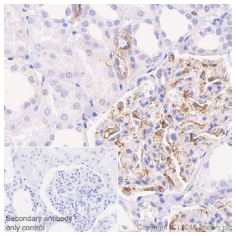
Recommended IHC antibody:
Recombinant Anti-EGFR antibody [SP84] - C-terminal (ab227642)

View antibodies to EGFR

Figure: Formalin-fixed, paraffin-embedded human colon adenocarcinoma tissue stained for EGFR using ab227642 at 1/100 dilution in immunohistochemical analysis.

VEGF

VEGF is a heparin-binding glycoprotein with potent angiogenic activity within endothelial cells. Angiogenesis is crucial for the progression of colorectal carcinoma. VEGF is expressed in around 50% of colorectal cancers and shows very low expression in normal colonic mucosa, making it a good biomarker for the diagnosis of CRC from histology samples[16].



IHC stain (marker localization):
Secreted protein

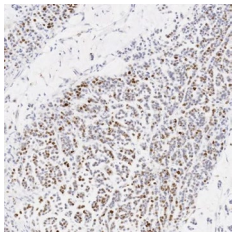
Recommended IHC antibody:
Recombinant Anti-VEGFA antibody [EP1176Y] - C-terminal (ab52917)

View antibodies to VEGF

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling VEGFA with purified ab52917 at 1:100 dilution (2.96 µg/mL).

p53

The tumor suppressor p53 is a transcription factor involved in cell cycle arrest and apoptosis under cellular stress. Mutations within p53 is one of the most frequent triggers leading to the progression of CRC and is found in 34% of the proximal colon tumors, and 45% of distal colorectal tumors.



IHC stain (marker localization):
Cytoplasm and strong nuclear

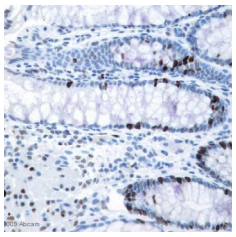
Recommended IHC antibody:
Recombinant Anti-p53 antibody [E26] (ab32389)

View antibodies p53

Figure: Formalin-fixed, paraffin-embedded human colon carcinoma tissue stained for p53 using ab16665 at 1/100 dilution in immunohistochemical analysis.

Ki67

Ki67 is an important protein involved in cell division and is commonly used as a marker of cellular proliferation. Quantifying Ki67 expression by IHC will give you the Ki67 labeling index, which is commonly used in clinical pathology to estimate cancer prognosis. High Ki67 expression is thought to be an indication of a good prognosis for patients with colorectal cancer.



IHC stain (marker localization):
Nuclear

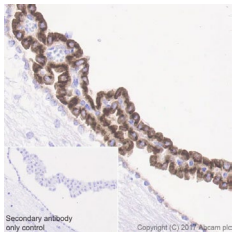
Recommended IHC antibody:
Recombinant Anti-Ki67 antibody [SP6] (ab16667)

View antibodies to Ki67

Figure: ab15580 staining Ki67-proliferation marker in human colon tissue sections by IHC-P (formaldehyde-fixed paraffin-embedded sections).

Insulin-like growth factor binding protein 2 (IGFBP2)

IGFBP-2 interacts with the extracellular matrix, proteoglycans and integrin receptors within many different cell types. It may also act as a transcription factor to stimulate gene expression. Studies have shown that IGFBP-2 is uniquely distributed at the bottom of human colonic crypts and that increased levels of IGFBP-2 can be seen in many colorectal cancers. This expression co-localizes with the phosphorylated p65 subunit of NF-kB, making these good biomarkers for CRC[17].



IHC stain (marker localization):
Secreted protein

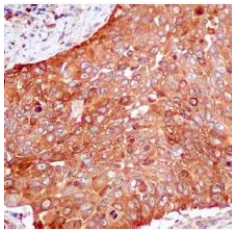
Recommended IHC antibody:
Recombinant Anti-IGFBP2 antibody [EPR18012-257] (ab188200)

View antibodies to IGFBP2

Figure: Immunohistochemical analysis of paraffin-embedded mouse choroid plexus tissue labeling IGFBP2 with ab188200 at 1/1000 dilution, followed by a Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining on mouse choroid plexus (PMID: 7525264; PMID: 7678219) is observed.

PKM2

The M2 isoform of PK (PKM2) is a glycolytic enzyme involved in aerobic glycolysis and anabolic metabolism in cancer cells. PKM2 also promotes the transcription of Oct-46, HIF-1α7, STAT38, and β-catenin during the progression of various cancers.



IHC stain (marker localization):
Nuclear and cytoplasm

Recommended IHC antibody:
Recombinant Anti-PKM antibody [EPR10139] (ab154816)

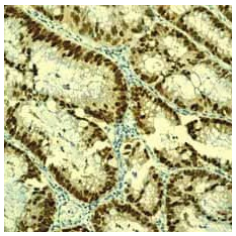
View antibodies to PKM2

Figure: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma, labeling PKM using ab154816 at 1/100 dilution.

Cell type specific biomarkers

CDX2

CDX2 is a transcription factor expressed specifically in the intestine, where it is involved in the maintenance of intestinal cell types. Low CDX2 expression in CRC is associated with advanced stages of cancer progression, vessel invasion, and metastasis. Over 20% of CRC show some reduction of CDX2 protein by histology. Seeing normal or high levels of CDX2 expression is a biomarker for a good prognosis for survival from the disease[18].



IHC stain (marker localization):
Nuclear

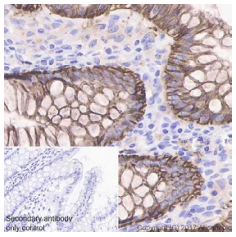
Recommended IHC antibody:
Recombinant Anti-CDX2 antibody [EPR2764Y] (ab76541)

View antibodies to CDX2

Figure: Unpurified ab76541 showing positive staining in colonic adenocarcinoma tissue. Heat-mediated antigen retrieval was performed before commencing with IHC staining protocol.

Ep-CAM

Ep-CAM is a commonly used biomarker used in colorectal adenocarcinoma to discriminate cancer cells from mesothelial cells. Being able to distinguish between adenocarcinoma and mesothelial cells by histology is crucial for clinicians to be able to correctly diagnose and assign treatment to patients.



IHC stain (marker localization):
Cell membrane

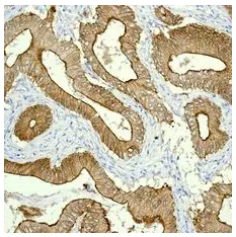
Recommended IHC antibody:
Recombinant Anti-EpCAM antibody [EPR20532-225] (ab223582)

View antibodies to Ep-CAM

Figure: Immunohistochemical analysis of paraffin-embedded human colon tissue labeling EpCAM with ab223582 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on human colon is observed (PMID: 15637741). Counterstained with hematoxylin.

Villin

Villin is an actin-binding protein expressed in the epithelial cells lining the gut and has been shown to regulate epithelial-mesenchymal transition (EMT) in colorectal cancers. Microsatellite instability (MSI) colorectal cancers have also been shown to have very low levels of villin expression compared to normal gut epithelial cells. This loss of villin leads to poorly differentiated histology of the CRC[19].



IHC stain (marker localization):
Cytoplasm

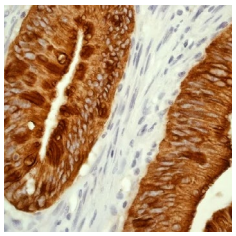
Recommended IHC antibody:
Recombinant Anti-Villin antibody [EPR3491(3)] (ab133510)

View antibodies to Villin

Figure: Immunohistochemical analysis of paraffin-embedded human colon adenocarcinoma tissue labeling villin with ab133510 at 1/100 dilution.

CK20 (Cytokeratin 20) and CK7 (Cytokeratin 7)

CK7 and CK20 are cytokeratins expressed in the gut epithelia. Different CK7 and CK20 IHC expression patterns are commonly used to distinguish colorectal adenocarcinomas. Most colorectal cancers are CK7 negative and CK20 positive, making the combination of these cytokeratins an excellent biomarker combination. CK7 and CK20 expression varies between colorectal carcinomas according to histological grade and tumor location.



CK20

IHC stain (marker localization):
Cytoplasm

Recommended CK20 IHC antibody:
Recombinant Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126)

View antibodies to CK20

Figure: ab76126 at 1/100 dilution staining Cytokeratin 20 in human colon adenocarcinoma by immunohistochemistry, paraffin-embedded tissue.

CK7

IHC stain (marker localization):
Cytoplasm

Recommended CK7 IHC antibody:
Recombinant Anti-Cytokeratin 7 antibody [EPR1619Y] - Cytoskeleton Marker (ab68459)

View antibodies to CK7

References

1. Flanagan, D. J., Pentinmikko, N., Luopajärvi, K. et al. NOTUM from Apc-mutant cells biases clonal competition to initiate cancer. *Nature* **594**, 430–435 (2021).

2. Vonlanthen, S., Kawecki,T. J., et al., Heterozygosity of SNP513 in Intron 9 of the Human Calretinin Gene (CALB2) is a Risk Factor for Colon Cancer A*NTICANCER RESEARCH* **27**, 4279-4288 (2007).

3. Gotzos V., Wintergerst E. S., et al., Selective distribution of calretinin in adenocarcinomas of the human colon and adjacent tissues. *Am J Surg Pathol* **23**(6), 701-11, (1999).

4. Winn B., Tavares, R., et al., Differentiating the undifferentiated: immunohistochemical profile of medullary carcinoma of the colon with an emphasis on intestinal differentiation. *Hum Pathol*, **40**(3), 398–404 (2009).

5. Betge, J., Schneider, N. I., et al., MUC1, MUC2, MUC5AC, and MUC6 in colorectal cancer: expression profiles and clinical significance. *Virchows Arch.*, **469**(3), 255–265 (2016).

6. Herendeen J. M., Lindley C., (2003). Use of NSAIDs for the chemoprevention of colorectal cancer. *Ann Pharmacother*; **37**(11):1664-74.

7. Sinicrope F. A., Gill S., Role of cyclooxygenase-2 in colorectal cancer. *Cancer Metastasis Rev*, **23**(1-2):63-75 (2004).

8. uniprot.org

9. Bartolomé, R. A., Barderas, R., et al., Cadherin-17 interacts with α2β1 integrin to regulate cell proliferation and adhesion in colorectal cancer cells causing liver metastasis. *Oncogene*, **33**: 1658–1669, (2013).

10. Heath J. K., White S. J., et al., The human A33 antigen is a transmembrane glycoprotein and a novel member of the immunoglobulin superfamily. *Proc Natl Acad Sci USA*; **94**(2): 469-74, (1997).

11. Baptistella, A. R., Salles Dias, M. V., et al., Heterogeneous expression of A33 in colorectal cancer: possible explanation for A33 antibody treatment failure. *Anticancer Drugs*, **27**(8): 734-7, (2016).

12. Cheal, S. M., Fung, E. K., et al., Curative Multicycle Radioimmunotherapy Monitored by Quantitative SPECT/CT-Based Theranostics, Using Bispecific Antibody Pretargeting Strategy in Colorectal Cancer. *J Nucl Med*, **58**:1735–1742 (2017).

13. Markman B., Javier Ramos F., et al., EGFR and KRAS in colorectal cancer *Adv Clin Chem*; **51**:71-119, (2010).

14. Bertotti, A., Papp, E., et al., The genomic landscape of response to EGFR blockade in colorectal cancer. *Nature*, **526**, 263–267, (2015).

15. Pabla, B., Bissonnette, M., and Konda,V. J., Colon cancer and the epidermal growth factor receptor: Current treatment paradigms, the importance of diet, and the role of chemoprevention. *World J Clin Oncol*, **6**(5): 133–141 (2015).

16. Bendardaf, Riyad et al. "VEGF-1 expression in colorectal cancer is associated with disease localization, stage, and long-term disease-specific survival." *Anticancer research*, **28**(6B), 3865-70 (2008).

17. Shmuel, B., Shvab, A., et al., Global analysis of L1-transcriptomes identified IGFBP-2 as a target of ezrin and NF-kB signaling that promotes colon cancer progression, *Oncogene*, **32**, 3220-3230, (2013).

18. Graule, J., Uth, K., et al., CDX2 in colorectal cancer is an independent prognostic factor and regulated by promoter methylation and histone deacetylation in tumors of the serrated pathway, *Clinical Epigenetics*, **10**(120), (2018).

19. Arango D, Al-Obaidi S, Williams DS, et al., Villin expression is frequently lost in poorly differentiated colon cancer. *Am J Pathol*, **180**(4):1509-21 (2012).

prostate cancer biomarkers

4

Prostate cancer biomarkers

Emerging prostate cancer biomarkers

Androgen Receptor (AR-V7 specific)

Although multiple androgen-receptor variants have been discovered, AR-V7 is the only known androgen-receptor variant encoding a functional protein product that is detectable in clinical specimens. Detection of AR-V7 in circulating tumor cells may be associated with resistance to enzalutamide and abiraterone in patients with castration-resistant prostate cancer[1].

View antibodies to AR-V7 specific

Hepsin/HPN

Hepsin may contribute to the poor response of prostate cancer cells to immunotherapies that rely on STING activation[2].

View antibodies to HPN

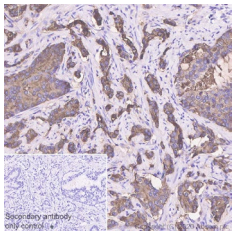
Prostate cancer is the second most common cancer in men worldwide. IHC is used for diagnosis from prostate biopsy, though the precision and accuracy of the current biomarkers used in this test remains controversial. Therefore, many are looking for alternative specific and sensitive prostate cancer biomarkers to improve outcomes[3]. While a panel of IHC prostate cancer biomarkers are commonly used for prostate cancer diagnosis (including PSA, AMACR and high molecular weight cytokeratins), issues around benign tumors mimicking this profile and the early detection of presence and progression of prostate cancer, and recurrence following clinical intervention are still not accurately validated by current biomarkers for prostate cancer. There remains a lack of reliable biomarkers to accurately predict low-risk cancer and avoid overtreatment.

As such, aggressive forms of prostate cancer may be missed, and indolent disease may be subjected to unnecessary radical therapy. New biomarker discovery and validation promise to improve early detection and prognosis and to provide targets for therapeutic interventions[4].

Established prostate cancer biomarkers

Prostate specific antigen (PSA)

PSA is the most widely known biomarker for prostate cancer. It is expressed in prostate epithelial cells and secreted to the seminal fluid, where it's responsible for cleaving semenogelins. PSA is considered to be a highly sensitive and specific biomarker for prostate tumors. It was approved by the FDA for diagnosis of prostate cancer in conjunction with digital rectal exam in 1994. Consequently, the detection of PSA in additional tissues via IHC is typically associated with metastatic cancer of prostatic origin, and the presence of this biomarker is often used to differentiate between prostatic and urothelial carcinomas[5].



IHC stain (marker localization):
Cytoplasmic and extracellular

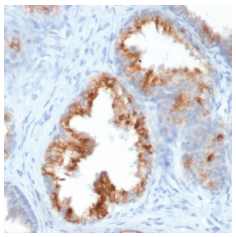
Recommended IHC antibody:
Recombinant Anti-Prostate Specific Antigen antibody [EP1588Y] (ab76113)

View antibodies to PSA

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate cancer tissue sections labeling Prostate Specific Antigen with purified ab76113 at 1/1000 dilution (0.51 µg/mL).

Alpha-methylacyl-CoA racemase (AMACR)

AMACR is a mitochondrial and peroxisomal enzyme that functions to oxidize fatty acids and bile acid intermediates. It is over-expressed in the epithelium of approximately 80% of prostate cancer cases and acts as a robust biomarker of prostate cancer. Detection of AMACR via IHC has been shown to be important in the differential diagnosis of prostate carcinoma from benign prostate mimics. AMACR overexpression in prostate cells is indicative of poor patient prognosis, associated with a high Gleason's score, high initial PSA levels, and indicative of the increased chance of bone metastasis[6].



IHC stain (marker localization):
Cytoplasmic

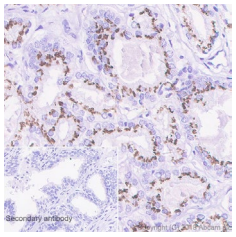
Recommended IHC antibody:
Anti-AMACR antibody [AMACR/1864] (ab268062)

View antibodies to AMACR

Figure: Formalin-fixed, paraffin-embedded human prostate carcinoma tissue stained for AMACR using ab268062 at 2 µg/mL in immunohistochemical analysis.

SLC45A3 (Prostate cancer-associated protein 6/P501S/prostein)

SLC45A3 shows a predominantly homogenous Golgi staining pattern in prostatic epithelia. It is highly specific for prostate glandular cells and thus used in the differentiation of metastatic prostate cancers from other tumor types. SLC45A3 may be expressed in PSA-negative prostate tumors, and the use of these markers in conjunction offers increased sensitivity in the identification of prostate cancer metastases[7]. Weaker expression of SLC45A3 has been noted in some aggressive tumors, correlating with increased Gleason scores and risk of cancer relapse.



IHC stain (marker localization):
Membrane

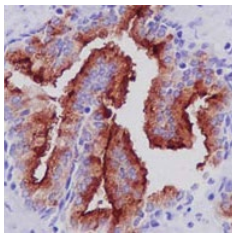
Recommended IHC antibody:
Recombinant Anti-SLC45A3 antibody [EPR4795(2)] (ab137065)

View antibodies to SLC45A3

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue sections labeling SLC45A3 with purified ab137065 at 1/1000 dilution (0.128 µg/mL).

Prostate specific membrane antigen (PSMA)

PSMA is a type II membrane glycoprotein with both folate hydrolase and N-acetylat-ed-alpha-linked-acidic peptidase (NAALAD) activity. In contrast to PSA, PSMA is expressed in high-grade prostatic adenocarcinomas and is associated with a high Gleason score. Detection of PSMA can help to identify metastatic prostate cancer in surgical specimens, yielding higher sensitivity than PSA alone[8]. The high sensitivity and specificity of this marker for prostatic carcinoma make it useful to differentiate prostate from urothelial tumors.



IHC stain (marker localization):
Cytoplasmic and cell membrane

Recommended IHC antibody:
Recombinant Anti-PSMA antibody [EPR6253] (ab133579)

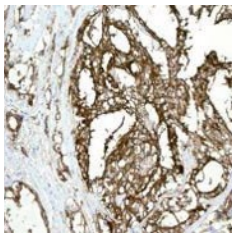
View antibodies to PSMA

Figure: Formalin-fixed, paraffin-embedded human prostate carcinoma tissue stained for PSMA using ab133579 at 1:300 in immunohistochemical analysis.

Prostatic acid phosphatase (PAP)

PAP is an acid phosphatase enzyme expressed in prostate tissue at levels approximately two-fold greater than in other tissues. This biomarker has been investigated as a marker for metastatic prostate cancer due to its high and relatively specific expression in prostate epithelial cells and has been considered as a target antigen in autologous cellular immunotherapy for patients with prostate cancer[9].

Knockdown of PAP expression is associated with promotion of cell growth and tumorigenicity, leading to the development of castration-resistant androgen-specific prostate cancer[10].



IHC stain (marker localization):
Secreted, cytoplasmic and cell membrane

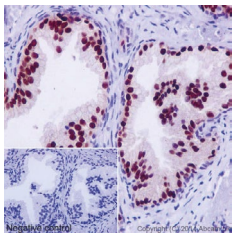
Recommended IHC antibody:
Recombinant Anti-PAP antibody [EPR4067] (ab109004)

View antibodies to PAP

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate tissue sections labeling PAP with purified ab109004 at 1/40,000 dilution (0.0024 µg/mL).

NKX3.1

NKX3.1 is a prostate-specific androgen-regulated transcription factor important in normal prostate development, regulating the proliferation of glandular epithelium, and in the formation of ducts in the prostate. Due to its highly specific expression in prostate epithelial cells, NKX3.1 can be used as a diagnostic biomarker for prostate cancer and other metastatic lesions originating in the prostate. Some studies show that NKX3.1 offers improved sensitivity over PSA for the identification of poorly differentiated metastatic prostate cancer[11].



IHC stain (marker localization):
Nuclear

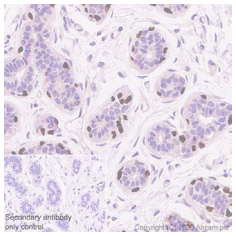
Recommended IHC antibody:
Recombinant Anti-NKX3 antibody [EPR16653] (ab196020)

View antibodies to NKX3.1

Figure: Formalin-fixed paraffin-embedded human prostate hyperplasia tissue stained for NKX3.1 (ab196020) at 1/500 dilution. Negative control: Used PBS instead of primary antibody.

p63

A member of the p53 protein family, p63 has pleiotropic functions, including cell proliferation, survival, apoptosis, differentiation, senescence and aging. This biomarker shows a nuclear localization in basal cells of the prostate and is absent from usual-type acinar prostate cancers. Aberrant p63 expression in prostate cancers may represent a molecularly distinct subclass and rare tumor type with some studies suggesting that further research of this biomarker may yield identification of the prostate cancer cell-of-origin[12].



IHC stain (marker localization):
Nuclear

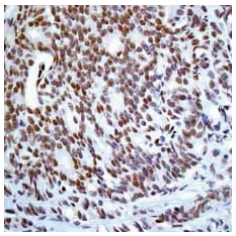
Recommended IHC antibody:
Recombinant Anti-p63 antibody [EPR5701] (ab124762)

View antibodies p63

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast tissue sections labeling p63 with purified ab124762 at 1/5000 dilution (0.16 µg/mL).

ERG

ERG is a transcriptional regulator that is identified in approximately half of all prostatic adenocarcinomas. This emerging biomarker for prostate cancer localizes to the nucleus of cells and is commonly found as a fusion product with TMPRSS2 or SLC45A3[13]. These ERG fusion products play an important role in the carcinogenesis of prostate cancer and may be predictive biomarkers of prostate cancer[14,15].



IHC stain (marker localization):
Nuclear and cytoplasmic

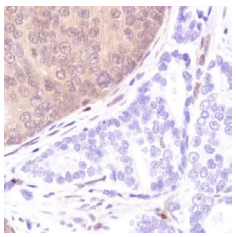
Recommended IHC antibody:
Recombinant Anti-ERG Antibody [EPR3864] (ab92513)

View antibodies to ERG

Figure: Formalin-fixed paraffin-embedded human prostatic adenocarcinoma stage 3 tissue stained for ERG (ab92513) using unpurified antibody in immunohistochemical analysis.

PTEN

PTEN protein loss is an emerging IHC biomarker for prostate cancer. This protein is a cytosolic lipid phosphatase that negatively regulates AKT activity in cell growth to act as a tumor suppressor. Loss of PTEN expression is observed in approximately 20-30% of cases and considered prognostic for poor patient outcomes in this disease[16].



IHC stain (marker localization):
Nuclear

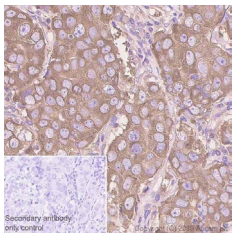
Recommended IHC antibody:
Recombinant Anti-PTEN Antibody [EPR9941-2] (ab170941)

View antibodies to PTEN

Figure: Formalin-fixed, paraffin-embedded human prostate adenocarcinoma tissue stained for PTEN using ab228466 at 1/200 dilution in immunohistochemical analysis.

Fatty acid synthase

This enzyme plays a role in the synthesis of long chain fatty acids. Fatty acid synthase overexpression is an emerging biomarker linked to prostate cancer carcinogenesis. Studies suggest that this emerging biomarker may act in cancer by inhibiting apoptosis, and associate Fatty acid synthase overexpression in prostate cancer IHC with increased Gleason score and more aggressive tumors[17,18].



IHC stain (marker localization):
Nuclear

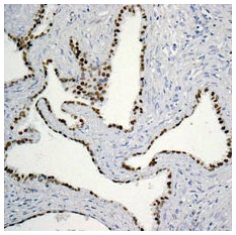
Recommended IHC antibody:
Recombinant Anti-Fatty Acid Synthetase Antibody (ab128870)

View antibodies to Fatty Acid Synthase

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast cancer tissue sections labeling Fatty acid synthase with purified ab128870 at 1:450 dilution (1.09 µg/mL).

FOXA1

Mutations in the FOXA1 transcription factor have been associated with tumor progression in prostate cancer. IHC analysis of FOXA1 as a prostate cancer biomarker has identified overexpression in prostate cancer metastases and linked this biomarker with more aggressive, castration-resistant cancer types. It may also act as an independent predictor of recurrence[19].



IHC stain (marker localization):
Nuclear

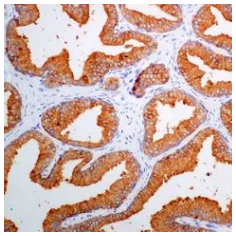
Recommended IHC antibody:
Recombinant Anti-FOXA1 antibody [EPR10881] - ChIP Grade (ab170933)

View antibodies to FOXA1

Figure: Formalin-fixed paraffin-embedded human prostate tissue labeled with FOXA1 (ab170933) with unpurified antibody at 1:100 in immunohistochemical analysis.

Prostate secretory protein 94 (PSP94)

PSP94 (prostate secretory protein of 94 aa; also called PIP) is one of the major secretory proteins from the prostate gland. This potential prostate cancer biomarker shows decreasing expression as prostate cancer progresses from a hormone-dependent to a hormone-independent state with a complete lack of PSP94 production in highly advanced metastatic prostate cancer. This differential expression could make PSP94 a prognostic clinical marker for prostate cancer and could help distinguish patients with aggressive forms of prostate cancer[20].



IHC stain (marker localization):
Cytoplasmic and secreted

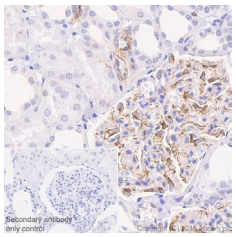
Recommended IHC antibody:
Recombinant Anti-Prostate Secretory Protein/PSP antibody [EPR7346] (ab133296)

View antibodies to PSP

Figure: Immunohistochemical analysis of paraffin-embedded human prostate tissue labeling Prostate Secretory Protein/PSP with 1/250 ab133296.

Vascular endothelial growth factor A (VEGFA)

VEGFA is a mediator of angiogenesis and tumor proliferation in many cancer types. Initial studies in prostate cancer suggest it may act as a prognostic biomarker for aggressive forms of prostate cancer and could be used to select prostate cancer patients suitable for novel anti-angiogenic therapies[21]. VEGFA expression has been shown to be significantly elevated in cases of prostatic cancer compared to benign hyperplasia, demonstrating it may serve as a potential diagnostic biomarker. Further, expression levels of VEGFA correlated with cancer grading, suggesting its utility as a prognostic marker[22].



IHC stain (marker localization):
Nuclear

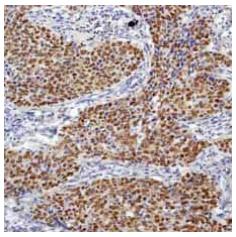
Recommended IHC antibody:
Recombinant Anti-VEGFA antibody [EP1176Y] - C-terminal (ab52917)

View antibodies to VEGFA

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling VEGFA with purified ab52917 at 1:100 dilution (2.96 µg/mL).

$p27^{kip1}$

In normal tissue, $p27^{kip1}$ is a tumor suppressor that inhibits cyclin/cyclin dependent kinase (CDK) cell cycle progression and regulates cancer cell invasion and migration. Decreased expression of $p27^{kip1}$ is associated with poor patient prognosis in prostate adenocarcinomas. Additionally, cytoplasmic localization of $p27^{kip1}$ has been linked to cyclin/CDK independent roles in tumorigenicity[23].



IHC stain (marker localization):
Nuclear and cytoplasmic in some cancers

Recommended IHC antibody:
Recombinant Anti-p27 KIP1 antibody [Y236] (ab32034)

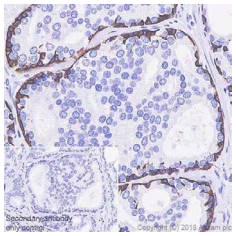
View antibodies to $p27^{kip1}$

Figure: Unpurified ab32034 showing positive staining in colonic adenocarcinoma tissue. Heat-mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).

Cell type specific biomarkers

Cytokeratin 5 (CK5)

CK5, basic (type II) cytokeratin, acts as a dimer in partnership with cytokeratin 14[24]. It is a member of the intermediate filament family of proteins, which provide the cell with a structural framework stretching from around the nucleus to desmosomes and hemidesmosomes. It is highly expressed in basal cells of prostate tissue, with diffuse cytoplasmic localization and perinuclear enhancement. This marker is used in conjunction with AMACR and PSA to rule out prostate cancer with increased accuracy[25].



IHC stain (marker localization):
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

View antibodies to CK5

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate cancer tissue sections labeling Cytokeratin 5 with ab64081 at 1/100 dilution (2.46 µg/mL). Heat-mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 10mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody.

Cytokeratin 6 (CK6)

CK6 is a basic cytokeratin that dimerizes with cytokeratin 16/17 for function[26]. CK6 is a cytoskeletal protein and serves as a marker for prostate basal cells, showing similar localization to CK5 of diffuse cytoplasmic staining with enrichment in the perinuclear area. Negative staining of this marker is often used in conjunction with CK5- and AMACR+ staining to diagnose problematic prostatic cancers[27].

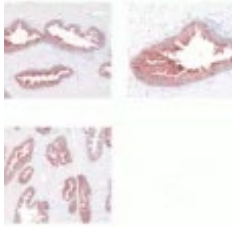
IHC stain (marker localization):
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-Cytokeratin 6 antibody [EP1603Y] (ab52620)

View antibodies to CK6

Kallikrein 2 (KLK2)

Kallikrein 2 is highly expressed in the prostate gland under androgen control for expression[28]. It is a trypsin-like serine protease requiring post-translational modification for activity. In prostate tissue, KLK2 functions upstream of PSA to activate this prostate cancer biomarker; consequently, it is associated with seminal plasma liquefaction[29].



IHC stain (marker localization):
Cytoplasmic and secreted

View antibodies to KLK2

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate tissue sections labeling Kallikrein with ab40749 at 18 µg/mL.

References

1. Emmanuel, S., et al. AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer *The New England Journal of Medicine*. **371**:1028-1038 (2014).

2. Fu, H., et al. The transmembrane serine protease hepsin suppresses type I interferon induction by cleaving STING. *Science Signaling*. **14**(687) (2021).

3. Jiang, N, Zhu S, Chen J et al.A-Methylacyl-CoA Racemase (AMACR) and Prostate-Cancer Risk: A Meta-Analysis of 4,385 Participants. *PLoS One* 2013; **8**(10): e74386.

4. Giannico GA, Arnold SA, Gellert LL and Hameed O. New and Emerging Diagnostic and Prognostic Immunohistochemical Biomarkers in Prostate Pathology. *Adv Anat Pathol*. 2017 Jan; **24**(1):35-44.

5. Saini, S. PSA and beyond: alternative prostate cancer biomarkers. *Cell Oncol* (Dordr). 2016 Apr; **39**(2): 97–106.

6. El Kassem, FA., Abulkheir, I., Sidom, N., et al. Role of immunohistochemical expression of AMACR as a prognostic and predictive biologic marker in advanced prostatic carcinoma. [http://dx.doi.org/10.1016/j.ejso.2016.06.190]

7. Sheridan T; Herawi M; Epstein JI; Illei PB (2007). The role of P501S and PSA in the diagnosis of metastatic adenocarcinoma of the prostate. *Am. J. Surg. Pathol*. **31**(9): 1351–5.

8. Bernacki KD, Fields KL and Roh MH. The utility of PSMA and PSA immunohistochemistry in the cytologic diagnosis of metastatic prostate carcinoma. *Diagn Cytopathol*. 2014 Jul; **42**(7):570-5.

9. Muniyan S, Chaturvedi NK, Dwyer JG et al. Human Prostatic Acid Phosphatase: Structure, Function and Regulation. *Int J Mol Sci*. 2013 May; **14**(5): 10438–10464.

10. Graddis TJ, McMahan CJ,Tamman J et al. Prostatic acid phosphatase expression in human tissues. *Int J Clin Exp Pathol*. 2011 Mar 31; **4**(3): 295–306.

11. Gurel B,Ali TZ, Montgomery EA, et al. NKX3.1 as a marker of prostatic origin in metastatic tumors. *Am J Surg Pathol*. 2010 Aug; **34**(8):1097-105.

12. Tan HL, Haffner MC, Esopi DM et al. Prostate adenocarcinomas aberrantly expressing p63 are molecularly distinct from usual-type prostatic adenocarcinomas. *Mod Pathol*. 2015 **28**(3):446-56.

13. Song C and Chen H. Predictive significance of TMRPSS2-ERG fusion in prostate cancer: a meta-analysis. *Cancer Cell International 2018* **18**(177).

14. Chaux A, Albadine R, Toubaji A , et al. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. *Am J Surg Pathol*. 2011 Jul; **35**(7):1014-20.

15. Andrews C and Humphrey PA. Utility of ERG versus AMACR expression in diagnosis of minimal adenocarcinoma of the prostate in needle biopsy tissue. *Am J Surg Pathol*. 2014 Jul; **38**(7):1007-12.

16. Geybels MS, Fang M,Wright JL, et al. PTEN loss is associated with prostate cancer recurrence and alterations in tumor DNA methylation profiles. *Oncotarget*. 2017 Oct 13; **8**(48): 84338–84348.

17. Migita T, Ruiz S, Fornari A, et al. Fatty Acid Synthase: A Metabolic Enzyme and Candidate Oncogene in Prostate Cancer. *J Natl Cancer Inst*. 2009 Apr 1; **101**(7):519-32.

18. Madigan AA, Rycyna KJ, Parwani AV, et al. Novel nuclear localization of fatty acid synthase correlates with prostate cancer aggressiveness. *Am J Pathol*. 2014 Aug;**184**(8):2156-62.

19. Gerhardt J, Montani M,Wild P, et al. FOXA1 promotes tumor progression in prostate cancer and represents a novel hallmark of castration-resistant prostate cancer. *Am J Pathol*. 2012 **180**(2):848-61.

20. Luebke AM,Attarchi-Tehrani A, Meiners J et al. Loss of PSP94 expression is associated with early PSA recurrence and deteriorates outcome of PTEN deleted prostate cancers. *Cancer Biol Med*. 2019;**16**(2):319-330.

21. Kamath A, Helie M, Bifulco CB, et al. Lack of immunohistochemical detection of VEGF in prostate carcinoma. *Appl Immunohistochem Mol Morphol*. 2009 **17**(3):227-32.

22. Gautama KA, Singh AN, Srivastavc AN, and Sankhwar AN. Angiogenesis in prostate cancer and benign prostatic hyperplasia assessed by VEGF and CD-34 IHC: A comparative clinico-pathological study. 2018 **24**(2):98-103.

23. Lee J & Kim SS. The function of p27KIP1 during tumor development. *Experimental & Molecular Medicine 41*, **765–771**(2009).

24. Pekny M, and Lane EB. Intermediate filaments and stress. *Exp Cell Res* 2007 **10**;313(10):2244-54.

25. Dabir PD, Ottosen P, Høyer S and Hamilton-Dutoit S Comparative analysis of three- and two-antibody cocktails to AMACR and basal cell markers for the immunohistochemical diagnosis of prostate carcinoma. *Diagn Pathol*. 2012 **16**;7:81.

26. Coulombe PA, Tong X, Mazzalupo S, et al. Great promises yet to be fulfilled: defining keratin intermediate filament function in vivo. *Eur J Cell Biol*. 2004 Dec; **83**(11-12):735-46.

27. Trpkov K, Bartczak-McKay J and Yilmaz A. Usefulness of cytokeratin 5/6 and AMACR applied as double sequential immunostains for diagnostic assessment of problematic prostate specimens. *Am J Clin Pathol*. 2009 **132**(2):211-20.

28. Chao J, Chen L, and Chai KX. Human Kallikrein-related Peptidase 2. In: *Handbook of Proteolytic Enzymes*. Vol. **3**, 2013, Pages 2762-2765.

29. Williams SA, Xu Y, De Marzo AM, Isaacs JT, and Denmeade SR. Prostate-Specific Antigen (PSA) Is Activated by KLK2 in Prostate Cancer Ex Vivo Models and in Prostate-Targeted PSA/KLK2 Double Transgenic Mice. *Prostate* 2010 15; **70**(7): 788–796.

ovarian cancer biomarkers

5

Ovarian cancer biomarkers

Emerging ovarian cancer biomarkers

CD9

CD9 is widely expressed in Tubo-ovarian high-grade serous carcinoma (HGSC) and represents an important new therapeutic target with immediate relevance for NK immunotherapy.

CD9 suppresses anti-tumor cytokine production and cytotoxicity in NK-92 cells[1].

View antibodies to CD9

PAX8

The transcription factor PAX8 is critical for the development of the thyroid and urogenital system. PAX8 binds a large number of genomic sites and forms transcriptional hubs. At a subset of these, PAX8 together with PRDM3 regulates a specific gene expression module involved in adhesion and extracellular matrix.

PRDM3 is amplified in ovarian cancers. The MECOM locus and PAX8 sustain *in vivo* tumor growth, further supporting that the identified function of the MECOM locus underlies PAX8-driven oncogenic functions in ovarian cancer[2].

View antibodies to PAX8

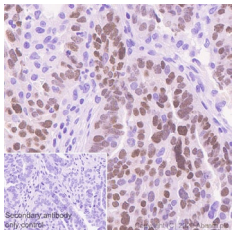
Ovarian cancer is the leading cause of gynecologic cancer death in women and impacts female life and health worldwide. The high death rate in this disease is due to the late stage of disease diagnosis. Although radical surgical tumor debulking and platinum plus paclitaxel-based chemotherapy are currently established therapies for the treatment of ovarian cancer, the 5-year survival rate is still around 40%[3]. The ability to sensitively and specifically predict the presence of early ovarian cancer, disease status, stage, and associated therapeutic efficacy, has the potential to revolutionize ovarian cancer detection and treatment[4].

Consequently, the identification and validation of applicable diagnostic and prognostic ovarian cancer biomarkers are essential to improve patient outcomes[3].

Established ovarian cancer biomarkers

Paired box 8 (PAX8)

PAX8 is a transcription factor essential in organogenesis, morphogenesis, cell growth, and differentiation. PAX8 is highly expressed in both benign and malignant primary epithelial ovarian carcinomas, but not in metastatic ovarian cancer[5]. High expression levels of PAX8 correlate with shorter survival rates. PAX8 acts as a diagnostic and prognostic biomarker for ovarian cancer[6].



IHC stain (marker localization):
Nuclear

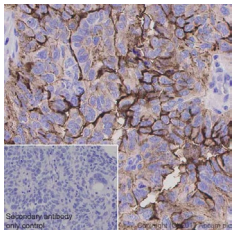
Recommended IHC antibody:
Recombinant Anti-PAX8 antibody [EPR23508-20] (ab239363)

View antibodies to PAX8

Figure: Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling PAX8 with ab239363 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining in human ovarian cancer (PMID: 21317881).

MUC16

Also known as CA125. This membrane-associated mucin, found in cornea and conjunctiva, in respiratory tract and female reproductive tract epithelium, forms a lubricating barrier against foreign particles and infectious agents. This biomarker is 79% sensitive for ovarian cancer and considered the most reliable diagnostic marker for ovarian cancer and a potential cancer therapeutic target. Assayed as a serum biomarker for ovarian cancer diagnosis, MUC16 is also used in IHC to distinguish ovarian origins of metastases[7].



IHC stain (marker localization):
Strong membranous staining

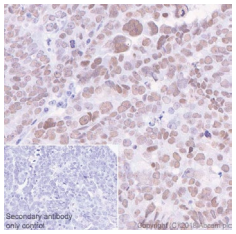
Recommended IHC antibody:
Recombinant Anti-MUC16 antibody [EPR1020(2)] (ab110640)

View antibodies to MUC16

Figure: FFPE IHC of human ovarian serous papillary carcinoma tissue sections labeling MUC16 with purified ab110640 at 1:1000 dilution (0.12 µg/mL). Negative control: PBS instead of the primary antibody.

Wilms tumor protein (WT1)

This ovarian cancer biomarker is used in IHC to differentiate ovarian carcinoma (WT1+) from breast or pancreatic carcinomas (WT1-). It is a robust diagnostic and prognostic biomarker of ovarian cancers and used in combination with PAX8 to refine diagnosis in phenotype overlapping cases of serous cancers[6,8].



IHC stain (marker localization):
Nuclear

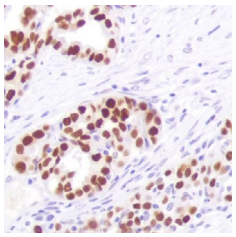
Recommended IHC antibody:
Recombinant Anti-WT1 antibody [CAN-R9(IHC)-56-2] (ab89901)

View antibodies to WT1

Figure: FFPE IHC of human ovarian serous adenocarcinoma tissue sections labeling Wilms Tumor Protein with Anti-WT1 (ab89901) at 1:500 dilution (0.49 µg/mL). Negative control: PBS instead of the primary antibody.

p53

This tumor suppressor protein is commonly mutated in many cancer types and overexpressed in over 96% of high-grade serous ovarian cancer. p53 mutations and p53 overexpression are both related to shorter patient survival, with the strongest predictor of outcome being a combination of both mutations and overexpression[9]. As an IHC biomarker, p53 expression is used to differentiate malignant cancers (p53+) from reactive and metaplastic conditions (p53-)[10].



IHC stain (marker localization):
Nuclear

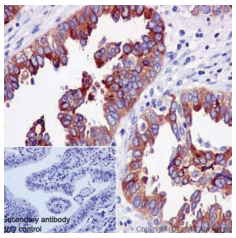
Recommended IHC antibody:
Recombinant Anti-p53 antibody [SP161] (ab227655)

View antibodies to p53

Figure: Formalin-fixed, paraffin-embedded human ovarian adenocarcinoma tissue stained for p53 using ab227655 at 1/100 dilution in immunohistochemical analysis.

Human epididymis protein 4 (HE4)

HE4 is a protease inhibitor that is approved by the United States FDA for monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. This biomarker is used in combination with MUC16 for the diagnosis of ovarian cancer, with some reports suggesting that HE4 offers increased specificity over MUC16 for the diagnosis of early ovarian cancer[11].



IHC stain (marker localization):
Nuclear

Recommended IHC antibody:
Recombinant Anti-HE4 antibody [EPR16658] (ab200828)

View antibodies to HE4

Figure: FFPE IHC of human ovarian carcinoma labeling HE4 with ab200828 at 1/1000 dilution.
Negative control: PBS instead of the primary antibody.

CDKN2A/p16INK4a

CDKN2a is a tumor suppressor protein, which, when silenced in ovarian cancer, can promote carcinogenesis. Increased expression of CDKN2A, the gene product of p16INK4a, in ovarian cancer has been associated with progression and unfavorable outcomes, though this may be histotype dependent, with some studies suggesting no overall association with survival in high-grade serous tumors, block expression of this biomarker associated with shorter overall survival in endometriosis-associated carcinomas, clear cell and endometrioid cancers, and absence of CDKN2A expression associated with shorter overall survival in low-grade serous ovarian carcinoma[12].

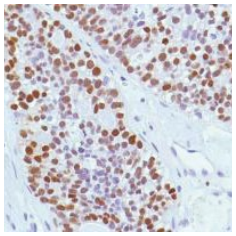
IHC stain (marker localization):
Nuclear and cytoplasmic

Recommended IHC antibody:
Recombinant Anti-CDNK2A/p16NK4a antibody [EPR1473] - C-terminal (ab108349)

View antibodies to CDKN2A/p16INK4a

Estrogen receptor alpha

Estrogen receptor alpha (ERα) is an established biomarker in the prognosis and treatment prediction of breast cancer. However, it is also emerging as a valuable biomarker in ovarian cancer. ERα staining in ovarian cancer IHC correlates with positive response to anti-estrogen treatment (e.g., tamoxifen) or chemotherapy, and better clinical outcomes. ERβ staining shows variable results dependent upon the stage and grade of ovarian cancer, with some studies showing negative expression correlates with favorable outcomes. ER expression in ovarian carcinoma is associated with better differentiated, more advanced tumors. ER levels are typically higher in high-grade, low-grade serous and endometrioid carcinoma, but lower in mucinous and clear-cell carcinoma[13].



IHC stain (marker localization):
Nuclear stain. Cytoplasmic staining alone is considered negative for ovarian cancer

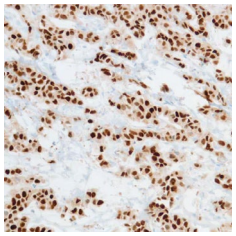
Recommended IHC antibody:
Anti-Estrogen Receptor alpha antibody [SP1] (ab16660)

View antibodies to Estrogen Receptor alpha

Figure: FFPE IHC of human ovarian adenocarcinoma tissue stained for Estrogen Receptor alpha using ab16660 at 1/200 dilution.

Progesterone receptor

This biomarker is often used in conjunction with estrogen receptor in IHC assay of ovarian carcinoma. Positivity of PR-B expression is correlated with positive response to chemotherapy and positive patient outcomes, though it offers limited additional information over ER alone[14].



IHC stain (marker localization):
Nuclear

Recommended IHC antibody:
Recombinant Anti-Progesterone Receptor [SP2] (ab16661)

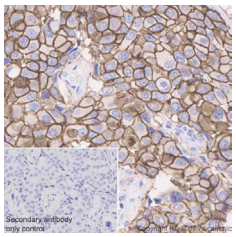
View antibodies to Progesterone Receptor

Figure: Immunohistochemistry analysis of human breast carcinoma tissue labeling SP2 with ab16661. This image was generated from the hybridoma version.

Human epidermal growth factor receptor 2 (HER2)

HER2 (also called c-erbB2, neu, ERBB2 and CD340) is a tyrosine kinase that dimerizes with members of the EGFR family for activity. Under normal conditions, HER2 acts to influence cellular migration, differentiation, and interactions between cells.

HER2 mutants offer a well-established breast cancer biomarker, but its use as an ovarian cancer biomarker is more controversial[15]. In ovarian cancer, HER2 expression is more commonly seen in the serous subtype, in older patients, patients with advanced stage and high-grade differentiation cancers, leading to poor patient prognosis for ovarian cancer patients[3].



IHC stain (marker localization):
Nuclear, perinuclear and membranous

Recommended IHC antibody:
Recombinant Anti-Erb2 antibody [EP1045Y] (ab134182)

View antibodies to HER2

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling EErbB2/HER2 with purified ab134182 at 1:1600 dilution (0.68 µg/mL).

Osteopontin

Osteopontin is a secreted, integrin-binding matrix phosphorylated glycoprotein that is overexpressed in many advanced cancers. It plays a role in many physiological and pathological processes; wound healing, inflammation, immune response, and tumorigenesis. Osteopontin promotes ovarian cancer progression and cell survival and increases HIF-1alpha expression through the PI3-K/Akt pathway. Osteopontin may serve as a potential diagnostic biomarker for ovarian cancer[16,17] and could potentially influence cancer therapy and be used in the development of novel anti-tumor treatments[17].

IHC stain (marker localization):
Secreted

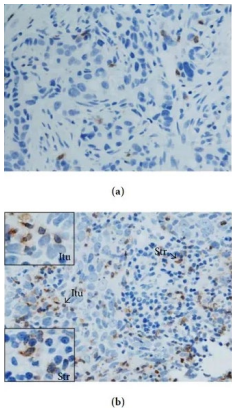
Recommended IHC antibody:
Recombinant Anti-Ostepontin antibody [EPR21139-316] (ab214050)

View antibodies to Osteopontin

Programmed cell death protein 1 (PD1)

Also called CD279. This well-known immune checkpoint receptor controls lymphocyte activation by providing negative signals in conjunction with signals from lymphocyte antigen receptors[18]. PD1 expression in ovarian cancers is associated with advanced stages of the disease in patients with high-grade tumors.

PD1 expression in IHC is an emerging biomarker for ovarian cancer diagnosis and poor patient prognosis. This biomarker is used to stratify patients for immuno-therapy with PD1 inhibitors, such as nivolumab, with the therapeutic effect seen only in a subset of patients, and additional biomarkers are required to accurately determine therapeutic efficacy[19,20].



IHC stain (marker localization):
Cell membrane

Recommended IHC antibody:
Recombinant Anti-PD1 antibody [CAL20] (ab237728)

View antibodies to PD1

Figure: Immunohistochemical analysis of human large and locally advanced breast cancers staining PD1 using ab52587. (Panel a) Low level of PD-1+ T cell infiltration. (Panel b) high level of PD-1+ T cell infiltration. (Itu: intratumoral Str: stromal).

Insulin-like growth factor binding protein 2 (IGFBP2)

Insulin-like growth factor binding protein 2 (IGFBP2) is overexpressed in malignant ovarian tissues and in the serum and cystic fluid of ovarian cancer patients. IGFBP2 overexpression does not correlate with the stage of ovarian cancer. This biomarker has shown some utility in the differentiation of serous carcinoma from clear cell carcinoma for ovarian cancer diagnosis[21]. IGFBP2 enhances the invasion capacity of ovarian cancer cells, consequently, blockage of IGFBP2 may thus constitute a viable strategy for targeted cancer therapy[22].

IHC stain (marker localization):
Secreted

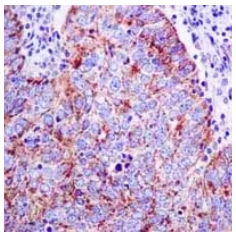
Recommended IHC antibody:
Recombinant Anti-IGFBP2 antibody [EPR18012-257] (ab188200)

View antibodies to IGFBP2

Kallikrein 8

This steroid-hormone regulated serine protease is not typically expressed in normal ovarian tissue, but elevated levels have been shown in ovarian cancers, suggesting this biomarker offers potential use in the diagnosis of ovarian cancer[23] .

Kallikrein 8-positive tumors have been associated with lower-grade tumors, no residual tumor after surgery, and optimal debulking success, suggesting Kallikrein 8 may act as a favorable prognostic biomarker for ovarian cancer[24].



IHC stain (marker localization):
Secreted

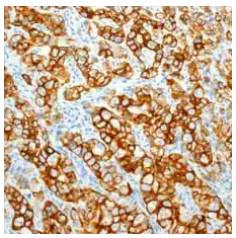
Recommended IHC antibody:
Recombinant Anti-Kallikrein 8/KLK8 antibody [EPR5752(2)] (ab150395)

View antibodies to KLK8

Figure: Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue labeling Kallikrein 8/KLK8 with ab150395 at 1/50 dilution.

Cytokeratin 7 (CK7)

CK7 is an established biomarker to differentiate primary ovarian carcinoma from metastatic colorectal carcinoma of the ovary. This biomarker can also be used in IHC analysis of ovarian cancer to differentiate primary serous tumors (negative) from primary mucinous tumors (positive). CK7 is negatively expressed in yolk sac tumors, but diffusely expressed in both clear cell carcinoma and endometrioid adenocarcinoma, making it a useful biomarker (often used in conjunction with EMA) to differentiate ovarian cancer subtypes[25,26].



IHC stain (marker localization):
Cytoplasmic

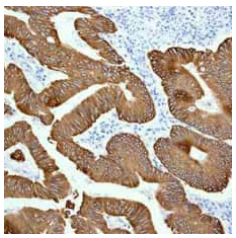
Recommended IHC antibody:
Recombinant Anti-Cytokeratin 7 antibody [EPR1619Y] - Cytoskeleton Marker (ab68459)

View antibodies to CK7

Figure: FFPE IHC of human ovarian carcinoma tissue stained for Cytokeratin 7 using ab68459.

Cytokeratin 20 (CK20)

CK20 is used as a biomarker in conjunction with CK7 to diagnose primary mucinous tumors of the ovary. Absence of this biomarker in CK7+ ovarian tumors is indicative of ovarian adenocarcinoma, including endometrioid, clear cell, serous and seromucinous cacrinomas. CK20 is also used in pathological analysis to discriminate between ovarian tumors and secondary metastatic tumors of the ovary[25,27].



IHC stain (marker localization):
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-Cytokeratin 20 antibody [EPR1622Y] - Cytoskeleton Marker (ab76126)

View antibodies to CK20

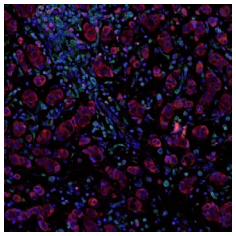
Figure: ab76126 showing positive staining in human colonic adenocarcinoma tissue.

Cell type specific biomarkers

Pan-cytokeratin

This cytokeratin cocktail recognizes K1 - 8, 10, 14 - 16 and 19, but does not detect CK17 or CK18. It is commonly used in IHC of ovarian tissue in conjunction with Vimentin.

Pan-cytokeratin staining shows membranous staining in the majority of tumor types and epithelial labeling in normal tissue. Pan-cytokeratin typically shows strong staining across healthy and tumorous tissue[28].



IHC stain (marker localization):
Cytoplasmic

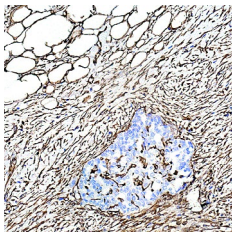
Recommended IHC antibody:
Recombinant Anti-pan Cytokeratin antibody [C-11] (ab7753)

View antibodies to Pan-CK

Figure: Fluorescence multiplex immunohistochemical analysis of human breast cancer tissue (formalin-fixed paraffin-embedded section). Merged staining of Anti-PD-L1 (ab251611; cyan; Opal™ 520), Anti-Granzyme B (ab219803; yellow; Opal™ 540), Anti-PD1 (ab251613; magenta; Opal™ 570), Anti-pan Cytokeratin (ab264485; red; Opal™ 620), Anti-EpCAM (ab225894; red; Opal™ 620), Anti-CD8 alpha (ab251596; green; Opal™ 650) and Anti-FOXP3 (ab96048; orange; Opal™ 690).

Vimentin

Vimentin is a type III intermediate filament protein that anchors the structure of the cytoplasm. In IHC of normal ovarian tissue, vimentin localizes to surface epithelium and granulosa cells. The variable staining of this biomarker in different ovarian carcinomas, from no staining in benign mucinous tumors or serous cystadenomas to strong cytoplasmic staining in malignant serous tumors, can be used to differentially diagnose various ovarian cancer types[28].



IHC stain (marker localization):
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

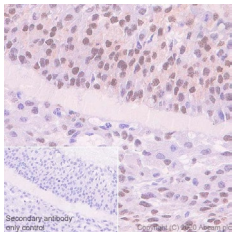
View antibodies to Vimentin

Figure: Anti-vimentin (ab92547) staining in human ovarian cancer tissue using immunohistochemistry (formaldehyde-fixed, paraffin-embedded sections).

Forkhead box L2 (FOXL2)

FOXL2 belongs to a large family of forkhead FOX transcription factors. It is one of the first genes expressed in female gonad development, required for proper granulosa cell differentiation during folliculogenesis, and maintains a strong expression in granulosa cells throughout life. FOXL2 mutations are present in 70 - 95% of ovarian adult granulosa cell tumors, but not in ovarian fibromas or ovarian juvenile granulosa cell tumors.

Consequently, IHC analysis of FOXL2 mutation is used by pathologists to distinguish diffuse adult granulosa cell tumors from cellular fibroma in ovarian tissue[29,30].



IHC stain (marker localization):
Nuclear

Recommended IHC antibody:
Recombinant Anti-FOXL2 antibody [EPR23523-68] (ab246511)

View antibodies to FOXL2

Figure: Immunohistochemical analysis of paraffin-embedded human ovary tissue labeling FOXL2 with ab246511 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human ovary.

ARID1A

This biomarker is a member of the SWI/SNF ATP-dependent chromatin-remodeling complexes[31]. It functions in cellular differentiation and development.
Expressed in the nucleus of normal tissue, inactivation of this protein and loss of expression is seen in ~50% of ovarian clear cell carcinoma[32] and may be an early event in the development of endometriosis-associated ovarian carcinomas[33,34].

IHC stain (marker localization):
Nuclear

Recommended IHC antibody:
Recombinant Anti-ARID1A antibody [EPR13501-73] (ab182561)

View antibodies to ARID1A

References

1. Gonzalez, V.D., et al. High-grade serous ovarian tumor cells modulate NK cell function to create an immune-tolerant microenvironment. *Cell reports*. **36**(9). 109632 (2021).

2. Bleu, M., Mermet-Meillon, F., Apfel, V. et al. PAX8 and MECOM are interaction partners driving ovarian cancer. *Nat Commun* **12**, 2442 (2021).

3. Luo H, Xu X, Ye M, et al. The prognostic value of HER2 in ovarian cancer: A meta-analysis of observational studies. *PLoS One*. 2018; **13**(1): e0191972.

4. Coticchia CM, Yang J, and Moses MA. Ovarian Cancer Biomarkers: Current Options and Future Promise. *J Natl Compr Canc Netw*. 2008 **6**(8): 795–802.

5. Chai HJ, Ren Q, Fan Q, et al. PAX8 is a potential marker for the diagnosis of primary epithelial ovarian cancer. *Oncol Lett*. 2017 Nov; **14**(5): 5871–5875.

6. Liliac L, Carcangiu ML, Canevari S, et al. The value of PAX8 and WT1 molecules in ovarian cancer diagnosis. *Rom J Morphol Embryol*. 2013; **54**(1):17-27.

7. Felder M, Kapur A, Gonzalez-Bosquet J, et al. MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. *Mol Cancer*. 2014; **13**:129.

8. Taube, Eliane Tabea et al. "Wilms tumor protein 1 (WT1) - not only a diagnostic but also a prognostic marker in high-grade serous ovarian carcinoma." *Gynecologic oncology vol*. **140**,3 (2016).

9. Cole AJ, Dwight T, Gill AJ, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. *Sci Rep*. 2016; **6**:26191.

10. McKenney JK, Desai S, Cohen C, and Amin MB. Discriminatory immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium: an analysis of cytokeratin 20, p53, and CD44 antigens. *Am J Surg Pathol*. 2001 Aug; **25**(8):1074-8.

11. Kalapotharakos G, Asciutto C, Henic E, et al. High preoperative blood levels of HE4 predicts poor prognosis in patients with ovarian cancer. *J Ovarian Res*. 2012; **5**: 20.

12. Rambau PF, Vierkant RA, Intermaggio MP, et al. Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an Ovarian Tumor Tissue Analysis consortium study. *J Pathol Clin Res*. 2018 **4**(4):250-261.

13. Chen S, Dai X, Gao Y, et al. The positivity of estrogen receptor and progesterone receptor may not be associated with metastasis and recurrence in epithelial ovarian cancer. *Sci Rep*. 2017 **7**(1):16922.

14. Lenhard M, Tereza L, Heublein S, et al. Steroid hormone receptor expression in ovarian cancer: progesterone receptor B as prognostic marker for patient survival. *BMC Cancer*. 2012 **12**:553.

15. Teplinsky E, and Muggia F. EGFR and HER2: is there a role in ovarian cancer? *Trans Cancer Res*. 2015 **4**(1):107-117.

16. Hu ZD, Weiwie TT, Yang M, et al. Diagnostic value of osteopontin in ovarian cancer: a meta-analysis and systematic review. *PLoS One*. 2015 **10**(5):e0126444.

17. Zhao H, Chen Q, Alam A, et al. The role of osteopontin in the progression of solid organ tumour. *Cell Death Dis*. 2018 **9**(3):356.

18. Thangavelu G, and Anderson CC. An essential role for programmed death-1 in the control of autoimmunity: implications for the future of hematopoietic stem cell transplantation. *Future Oncol*. 2011 Aug; **7**(8):929-32.

19. Drakes NL, Mehrotra S, Aldulescu M, et al. Stratification of ovarian tumor pathology by expression of programmed cell death-1 (PD-1) and PD-ligand- 1 (PD-L1) in ovarian cancer. *J Ovarian Res*. 2018; **11**:43.

20. Xue C, Zhu D, Chen L, et al. Expression and prognostic value of PD-L1 and PD-L2 in ovarian cancer. *Trans Cancer Res*. 2019 **8**(1):111-119.

21. Pickard A, and McCance DJ. IGF-Binding Protein 2 - Oncogene or Tumor Suppressor? *Front Endocrinol (Lausanne)*. 2015 **6**:25.

22. Lee EJ, Mircean C, Shmulevich I, et al. Insulin-like growth factor binding protein 2 promotes ovarian cancer cell invasion. *Mol Cancer*. 2005 **4**(1):7.

23. Kishi T, Grass L, Soosaipillai A, et al. Human kallikrein 8, a novel biomarker for ovarian carcinoma. *Cancer Res*. 2003 **63**(11):2771-4.

24. Borgoño CA, Kishi T, Scorilas A, et al. Human kallikrein 8 protein is a favorable prognostic marker in ovarian cancer. *Clin Cancer Res*. 2006 **12**(5):1487-93.

25. Ramalingam P, Malpica A, Silva EG, et al. The use of cytokeratin 7 and EMA in differentiating ovarian yolk sac tumors from endometrioid and clear cell carcinomas. *Am J Surg Pathol*. 2004 **28**(11):1499-505.

26. Vang R, Gown AM, Barry TS, et al. Cytokeratins 7 and 20 in primary and secondary mucinous tumors of the ovary: analysis of coordinate immunohistochemical expression profiles and staining distribution in 179 cases. *Am J Surg Pathol*. 2006 **30**(9):1130-9.

27. Taylor J, and McCluggage WG. Ovarian seromucinous carcinoma: report of a series of a newly categorized and uncommon neoplasm. *Am J Surg Pathol*. 2015 Jul; **39**(7):983-92.

28. Goel A, Mohan Rao N, Santhi V, et al. Immunohistochemical Characterization

of Normal Ovary and Common Epithelial Ovarian Neoplasm with a Monoclonal Antibody to Cytokeratin and Vimentin. *Iran J Pathol*. 2018; **13**(1): 23-29.

29. Kommoss S, Anglesio MS, Mackenzie R, et al. FOXL2 molecular testing in ovarian neoplasms: diagnostic approach and procedural guidelines. *Mod Pathol*. 2013 **26**(6):860-7.

30. McCluggage WG, Singh N, Kommoss S, et al. Ovarian cellular fibromas lack FOXL2 mutations: a useful diagnostic adjunct in the distinction from diffuse adult granulosa cell tumor. *Am J Surg Pathol*. 2013 **37**(9):1450-5.

31. Mao TL, Ardighieri L, Ayhan A, Kuo KT, Wu CH, Wang TL, Shih IeM. Loss of ARID1A expression correlates with stages of tumor progression in uterine endometrioid carcinoma. *Am J Surg Pathol*. 2013 Sep; **37**(9):1342-8.

32. Huang HN, Lin MC, Huang WC, et al. Loss of ARID1A expression and its relationship with PI3K-Akt pathway alterations and ZNF217 amplification in ovarian clear cell carcinoma. *Mod Pathol*. 2014 **27**(7):983-90.

33. Samartzis EP, Noske A, Dedes KJ, et al. ARID1A mutations and PI3K/AKT pathway alterations in endometriosis and endometriosis-associated ovarian carcinomas. *Int J Mol Sci*. 2013 **14**(9):18824-49.

34. Xiao W, Awadallah A, and Xin W. Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. *Int J Clin Exp Pathol*. 2012 **5**(7):642-50.

pancreatic cancer biomarkers

6

Pancreatic cancer biomarkers

Emerging pancreatic cancer biomarkers

TFF1

TFF1 has been used for the detection of pancreatic ductal adenocarcinoma in urine samples[1].

View antibodies to TFF1

Pentraxin 3/PTX3

PTX3 is a stromal compartment-specific pancreatic cancer biomarker that is suitable for validation studies[2].

View antibodies to PTX3

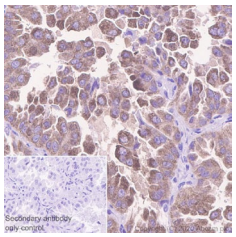
Pancreatic cancer is the 7th leading cause of cancer-related death worldwide with a higher death toll in developed countries. The most common subtype of pancreatic cancer is pancreatic ductal adenocarcinoma, accounting for approximately 85% of all cases. Globally, the mortality rate coincides with the incidence rate, emphasizing the poor prognosis for this cancer type[3]. Immunohistochemistry offers a useful assay in the identification and classification of pancreatic neoplasms.

The diagnostic accuracy for this cancer type has been significantly improved by the continuous discovery and validation of new tumor-associated biomarkers and the development of effective immunohistochemical panels. Application of appropriate IHC panels allows pathologists to differentiate between the different types of pancreatic cancer and to distinguish pancreatic carcinomas from other secondary metastatic cancers[4].

Established pancreatic cancer biomarkers

Insulin-like growth factor 2 binding protein 3 (IMP3)

IMP3, a cell-surface glycoprotein, is not expressed in normal pancreatic ductal epithelium and so may serve as a sensitive and specific biomarker to discriminate between benign and malignant pancreatic epithelium[5]. This biomarker may play a role in the migration, invasion and adhesion of pancreatic cell cancer. Identification of IMP3 by IHC is associated with poor prognosis of pancreatic ductal adenocarcinoma[6].



IHC stain:
Nuclear and cytoplasmic

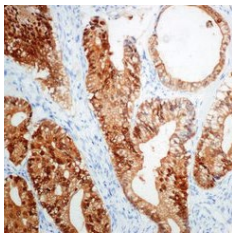
Recommended IHC antibody:
Recombinant Anti-IMP3 anitbody [ERP12021-114] (ab179807)

View antibodies to IMP3

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung cancer tissue sections labeling IMP3 with purified ab179807 at 1/1000 dilution (0.10 µg/mL).

S100 calcium binding protein P (S100P)

S100P is a sensitive and specific diagnostic biomarker for pancreatic cancer. It has a potential role in the proliferation, survival, motility and invasiveness of pancreatic cells. Levels of S100P expression increase during the progression from pancreatic intraepithelial neoplasia to invasive adenocarcinoma. This biomarker can be used to differentiate between positive pancreatic adenocarcinoma and negative pancreatic endocrine tumors[7].



IHC stain:
Nuclear and cytoplasmic

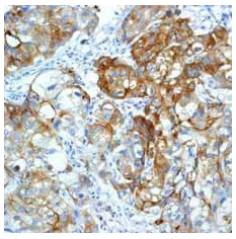
Recommended IHC antibody:
Recombinant Anti-S100P antibody [EPR6143] (ab133554)

View antibodies to S100P

Figure: Formalin-fixed paraffin-embedded human pancreatic adenocarcinoma tissue labeled with Anti-S100P (ab133554) at 1:250 in IHC.

MUC1

Also known as Epithelial Membrane Antigen (EMA), CD227 and episialin.
In normal cells, MUC1 acts as a barrier to the apical surface of epithelial cells, playing a protective and regulatory role. In pancreatic adenocarcinoma, MUC1 is highly expressed and associated with poor patient prognosis[8].



IHC stain:
Membranous and cytoplasmic

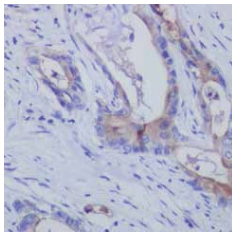
Recommended IHC antibody:
Recombinant Anti-MUC1 antibody [EPR1023] (ab109185)

View antibodies to MUC1

Figure: ab109185 (unpurified) showing positive staining in breast ductal infiltrating carcinoma tissue.

Mesothelin (MSLN)

MSLN is expressed on the surface of pancreatic adenocarcinoma cells and may play a role in cell adhesion. It serves as a diagnostic and prognostic biomarker for pancreatic cancer and may be used to target immunotherapy. Mesothelin expression in pancreatic adenocarcinoma is associated with high tumor aggressiveness and poor patient outcome[9].



IHC stain:
Extracellular, cell membrane and punctate cytoplasmic staining

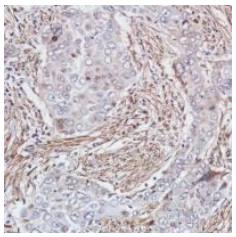
Recommended IHC antibody:
Recombinant Anti-Mesothelin antibody [EPR2685(2)] (ab134109)

View antibodies to MSLN

Figure: Formalin-fixed paraffin-embedded human pancreatic carcinoma labeled with Anti-Mesothelin (ab134109) using unpurified antibody in IHC analysis.

SMAD4

Also known as DPC4 and MADH4. SMAD4 acts downstream of TGF-β as a tumor suppressor through inhibiting growth and promoting apoptosis. Loss of function of SMAD4 may therefore act as a tumorigenic factor. Loss of SMAD4 expression in IHC has been associated with poor survival and may represent a negative prognostic biomarker in patients with pancreatic cancer[10].



IHC stain:
Nuclear staining in normal cells with loss of nuclear staining in malignancy

Recommended IHC antibody:
Recombinant Anti-SMAD4 antibody [SP306] - C-terminal (ab217267)

View antibodies to SMAD4

Figure: Formalin-fixed paraffin-embedded human pancreatic adenocarcinoma labeled with Anti-SMAD4 (ab217267) at 1:100 in IHC.

Toposiomerase II alpha (TOP2A)

TOP2A cleaves the double-stranded DNA helix in protein synthesis and DNA replication. Overexpression of this enzyme induces tumor development and progression[11]. TOP2A over-expression is correlated with tumor metastasis and shorter patient survival times, thus TOP2A is considered a prognostic biomarker in pancreatic cancer[12].

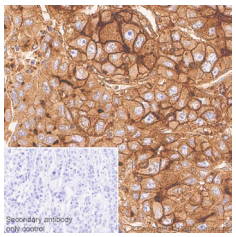
IHC stain:
Cytoplasmic and nucleoplasmic

Recommended IHC antibody:
Recombinant Anti-Toposiomerase II alpha anitbody [EP1102Y] (ab52934)

View antibodies to TOP2A

CEACAM1

Also called CD66, biliary glycoprotein, BGP and C-CAM. CEACAM1 is a cell adhesion molecule expressed in adenocarcinomas and in pancreatic intraductal neoplasia lesions[13]. Initially regarded as a tumor suppressor as its expression shows considerable downregulation within the epithelia in the early phases of solid cancers, more recently CEACAM1 has been suggested as a biomarker for the progression of malignancy and metastatic spread[14].



IHC stain:
Secreted and cell membranous

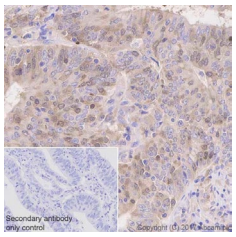
Recommended IHC antibody:
Recombinant Anti-CEACAM1 antibody [EPR4049] (ab108397)

View antibodies to CEACAM1

Figure: Immunohistochemical analysis of paraffin-embedded human hepatic carcinoma tissue labeling CEACAM1 with ab300061 at 1/2000 followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human hepatic carcinoma (PMID: 27377843).

Thymidylate synthase

Under normal conditions, this enzyme contributes to the *de novo* mitochondrial thymidylate biosynthesis pathway. It is the target for 5-fluorouracil (5-FU) in the treatment of pancreatic cancer. Thymidylate synthase has been identified as a prognostic biomarker for favorable outcomes following resection in patients with pancreatic adenocarcinoma[15].



IHC stain:
Cytoplasmic

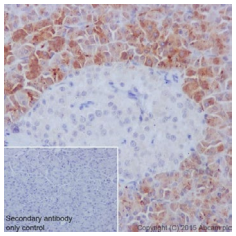
Recommended IHC antibody:
Recombinant Anti-Thymidylate Synthase antibody [EPR4545] (ab108995)

View antibodies to Thymidylate Synthase

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon carcinoma tissue sections labeling Thymidylate Synthase with purified ab108995 at 1:100 dilution.

Regenerating gene protein 3A (REG3A)

This pancreatic protein is a calcium-dependent protein that promotes islet growth in response to inflammation or injury. REG3A is an emerging biomarker for pancreatic cancer development. Its role in pancreatic cancer has been linked to inflammation-related pancreatic cancer development. High REG3A expression levels in IHC are common in the early stages of pancreatic cancer and may act as ancillary diagnostic and prognostic factors for the development of pancreatic ductal adenocarcinoma[16].



IHC stain:
Nuclear

Recommended IHC antibody:
Recombinant Anti-REG3A + REG3G antibody [EPRR18188] (ab202057)

View antibodies to REG3A

Figure: Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling REG3G + REG3A with ab202057 at 1/600 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

p-glycoprotein

P-glycoprotein acts as an ATP-dependent drug efflux pump to remove drugs and toxins from cells. Pancreatic tumors and ductal adenocarcinomas are both associated with elevated expression levels of p-glycoprotein. Expression levels of this novel pancreatic cancer biomarker correlate with a more favorable patient prognosis[17].

IHC stain:
Cell membrane

Recommended IHC antibody:
Recombinant Anti-P glycoprotein antibody [EPR10364-57] (ab170904)

View antibodies to p-glycoprotein

KRAS

This G-protein acts downstream of the Epidermal Growth Factor Receptor, and a single amino acid substitution results in an activating mutation. In pancreatic cancer, KRAS mutations, such as G12D, occur early during carcinogenesis, are present in 90% of tumors and associated with poor prognosis[18]. These mutations are used to differentiate between metastatic pancreatic cancer and primary tumors in other mucinous tissues.

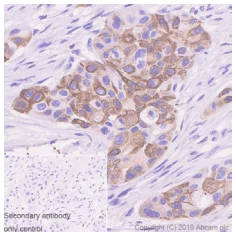
IHC stain:
Cell membrane

Recommended IHC antibody:
Recombinant Anti-Ras antibody [EPR23474-20] (ab275875)
Recombinant Anti-Ras (mutated G12D) antibody [HL-10] – BSA and Azide free (ab289373)

View antibodies to KRAS

MUC4

This transmembrane mucin protein provides a protective layer of mucus to the apical surface of epithelial cells. MUC4 is expressed in pancreatic ductal adenocarcinoma, but not in normal pancreatic tissue. In pancreatic cancer, it has a proposed role in cancer progression and metastasis[19].



IHC stain:
Secreted and cell membrane

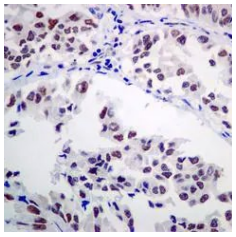
Recommended IHC antibody:
Recombinant Anti-MUC4 antibody [SP241] - C-terminal (ab183320)

View antibodies to MUC4

Figure: Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human pancreatic carcinoma tissue sections labeling MUC4 with ab183320 at 1/100 dilution (0.49 µg/mL).

Excision repair cross-complementation group 1 (ERCC1)

ERCC1 is an emerging biomarker used in guiding the treatment of pancreatic cancer. It functions in DNA repair, thus playing a role in the resistance to radiation and platinum-based therapies. IHC localization of ERCC1 has, in some cases, been associated with a positive response to chemotherapeutic agents for the treatment of pancreatic cancer[20].



IHC stain:
Nuclear

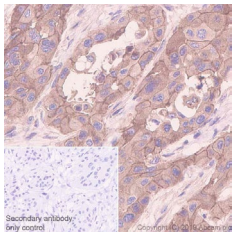
Recommended IHC antibody:
Recombinant Anti-ERCC1 antibody [EPR7277] (ab129267)

View antibodies to ERCC1

Figure: Formalin-fixed, paraffin-embedded human lung carcinoma tissue stained for ERCC1 with ab129267 (1/100 dilution) in immunohistochemical analysis.

Epidermal growth factor receptor (EGFR)

Also called HER1 and c-erb-B1. A member of the tyrosine kinase family of growth factors receptors, EGFR shows over-expression in pancreatic cancers by IHC. Increased EGFR expression in prostate IHC correlates with more advanced disease, poorer patient survival and the presence of metastases, thus EGFR inhibition has been an attractive focus for therapeutic intervention in pancreatic cancer[21].



IHC stain:
Cell membrane

Recommended IHC antibody:
Recombinant Anti-EGFR antibody [E234] (ab32198)

View antibodies to EGFR

Figure: FFPE IHC of human pancreatic carcinoma tissue sections labeling EGFR with purified ab32198 at 1/100 dilution (2.22 µg/mL). Negative control: PBS instead of the primary antibody.

Topoisomerase I

This enzyme is essential for genomic stability, acting to remove DNA supercoils. Topoisomerase I has been targeted by many inhibitors as a therapeutic strategy in the treatment of pancreatic cancer. Overexpression of Topoisomerase I is found in approximately half of all pancreatic cancers. It has been used as an IHC biomarker in many clinical studies to monitor patient response to pancreatic cancer therapies and as an emerging biomarker in monitoring the development of this cancer[22].

IHC stain:
Nuclear

Recommended IHC antibody:
Recombinant Anti-Topoisomerase I antibody [EPR5375] (ab109374)

View antibodies to Topoisomerase I

RRM1

RRM1 catalyzes the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides for DNA synthesis. IHC assay of RRM1 in pancreatic cancer has been used to predict patient response to chemotherapy[23]. High expression levels of RRM1 in pancreatic cancer may serve as a favorable prognostic biomarker[24].

IHC stain:
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-RRM1 antibody [EPR8483] (ab137114)

View antibodies to RRM1

Claudin 4

Claudin 4 is overexpressed in the majority of pancreatic carcinomas, both in early-stage lesions and advanced tumors. IHC assay of Claudin 4 offers targeting for novel therapeutics of infiltrating pancreatic cancer. Identification of Claudin 4 in the precursor lesions of pancreatic cancer suggests a potential benefit of IHC assay for Claudin 4 as both early diagnostic and prognostic of pancreatic cancer[25].

IHC stain:
Cell membrane

Recommended IHC antibody:
Recombinant Anti-Claudin 4 antibody [EPRR17575] (ab210796)

View antibodies to Claudin 4

Cell type specific biomarkers

Immunoglobulin G4 (IgG4)

This is the least abundant of the IgG subclasses, representing just 6% of IgG. It is commonly expressed in the pancreatobiliary region[26] and is used in the diagnosis of pancreatic cancer to distinguish between potential carcinoma and sclerotic pancreatic tissue in cases of autoimmune disorders[27].

IHC stain:
Cytoplasmic

Recommended IHC antibody:
Recombinant Anti-IgG4 antibody [EP4420] (ab109493)

View antibodies to IgG4

FOXA2

FOXA2 is a winged-helix transcription factor that functions to promote differentiation of pancreatic glucagon-producing alpha-cells and differentiation and maintenance of insulin-producing beta-cell secretory and metabolic pathways[28,29]. This pancreatic tissue biomarker is expressed in both normal and pancreatic ductal adenocarcinomas and may be used to identify pancreatic tissue and metastatic cancers[30].

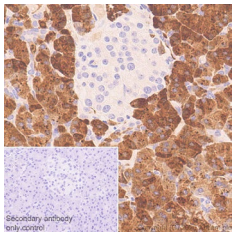
IHC stain:
Nuclear

Recommended IHC antibody:
Recombinant Anti-FOXA2 antibody [EPR4466] (ab108422)

View antibodies to FOXA2

Carboxypeptidase A1

This pancreatic metalloprotease is expressed in pancreatic juice and acts as a digestive enzyme on aromatic and aliphatic amino acid residues exposed by the action of chymotrypsins and elastases. Mutations in this enzyme are commonly associated with pancreatitis, while over-expression may be linked to pancreatic cancers, allowing diagnostic differentiation via IHC between these two conditions[31].



IHC stain:
Secreted

Recommended IHC antibody:
Recombinant Anti-Carboxypeptidase A antibody [EPR24384-69] (ab278044)

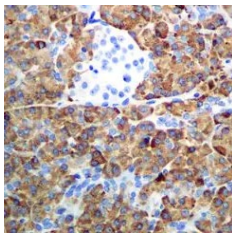
View antibodies to Carboxypeptidase A1

Figure: Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling Carboxypeptidase A with ab278044 at 1/4000 (0.135 µg/mL) dilution followed by a ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Pancreatic triacylglycerol lipase

Pancreatic triacylglycerol lipase is secreted by the pancreas and hydrolyzes triglycerides in the small intestine. This enzyme is essential for the efficient digestion of dietary fats.

Inhibition of pancreatic triacylglycerol lipase may prevent high-fat diet-induced obesity in mice and result in weight loss in human patients with obesity. Variants of this protein are commonly used in IHC as a biomarker for pancreatitis and may be used to identify pancreatic tissue[32].



IHC stain:
Secreted

Recommended IHC antibody:
Recombinant Anti-Pancreatic Lipase/PTL antibody [EPR6275] (ab133556)

View antibodies to PTL

Figure: Immunohistochemical analysis of paraffin-embedded human pancreas tissue labeling Pancreatic Lipase/PTL with ab133556 antibody at a dilution of 1/100.

References

1. Debernardi, S., et al. A combination of urinary biomarker panel and PancRISK score for earlier detection of pancreatic cancer: A case–control study. *PLOS MEDICINE*. (2020).

2. Watt, J., et al. Role of PTX3 in pancreatic cancer. *THE LANCET*. **383**(1). S106 (2014).

3. Hasan S, Jacob R, Manne U, and Paluri R. Advances in pancreatic cancer biomarkers. *Oncol Rev*. 2019 **13**(1): 410.

4. Lin F, Chen ZE, and Wang HL. Utility of immunohistochemistry in the pancreatobiliary tract. *Arch Pathol Lab Med*. 2015 **139**(1):24-38.

5. Han L, and Patel C. Utility of IMP3 Immunohistochemistry in the Distinction of Pancreatic Adenocarcinoma and Chronic Pancreatitis. *American Journal of Clinical Pathology* 2014 **142**(1) A225.

6. Pasiliao CC, Chang CW, Sutherland BW, et al. The involvement of insulin-like growth factor 2 binding protein 3 (IMP3) in pancreatic cancer cell migration, invasion, and adhesion. *BMC Cancer*. 2015 **15**:266.

7. Hu H, Zhang Q, Huang C, Shen Y, Chen X, Shi X, Tang W. Diagnostic value of S100P for pancreatic cancer: a meta-analysis. *Tumour Biol*. 2014; **35**(10):9479-85.

8. Nath S, Daneshvar K, Roy LD et al. MUC1 induces drug resistance in pancreatic cancer cells via upregulation of multidrug resistance genes. *Oncogenesis*. 2013 **17**; 2:e51.

9. Inaguma S, Wang Z, Lasota J, et al. Comprehensive immunohistochemical study of mesothelin (MSLN) using different monoclonal antibodies 5B2 and MN-1 in 1562 tumors with evaluation of its prognostic value in malignant pleural mesothelioma. *Oncotarget*. 2017 **8**(16): 26744–26754.

10. Shugang X, Hongfa Y, Jianpeng L et al. Prognostic Value of SMAD4 in Pancreatic Cancer: A Meta-Analysis. *Transl Oncol*. 2016 **9**(1): 1–7.

11. Pei Y, Yin X, and Liu X. TOP2A induces malignant character of pancreatic cancer through activating β-catenin signaling pathway. *Biochim Biophys Acta Mol Basis Dis*. 2018 **1864**(1):197-207.

12. Zhou T, Wang Y, Qian S, et al. Over-expression of TOP2A as a prognostic biomarker in patients with glioma. *Int J Clin Exp Pathol*. 2018; **11**(3): 1228–1237.

13. Simeone DM, Ji B, Banerjee M, Arumugam T, et al. CEACAM1, a novel serum biomarker for pancreatic cancer. *Pancreas*. 2007 **34**(4):436-43.

14. Calinescu A, Turcu G, Nedelcu RI, et al. On the Dual Role of Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1 (CEACAM1) in Human Malignancies. *J Immunol Res*. 2018 2018:7169081.

15. Guo YM, Zhu M, and Yu WW. Prognostic significance of thymidylate synthase expression in pancreatic adenocarcinoma: A meta-analysis. *Mol Clin Oncol*. 2015 **3**(1): 121–124.

16. Liu X, Wang J, and Wang H et al. REG3A accelerates pancreatic cancer cell growth under IL-6-associated inflammatory condition: Involvement of a REG3A-JAK2/STAT3 positive feedback loop. *Cancer Lett*. 2015 **362**(1):45-60.

17. Suwa H, Ohshio G, Arao S, et al. Immunohistochemical localization of P-glycoprotein and expression of the multidrug resistance-1 gene in human pancreatic cancer: relevance to indicator of better prognosis. *Jpn J Cancer Res*. 1996; **87**(6):641-9.

18. Lee J, Jang KT, Ki CS, et al. Impact of epidermal growth factor receptor (EGFR) kinase mutations, EGFR gene amplifications, and KRAS mutations on survival of pancreatic adenocarcinoma. *Cancer*. 2007 **109**(8):1561-9.

19. Ansari D, Urey C, Gundewar C, et al. Comparison of MUC4 expression in primary pancreatic cancer and paired lymph node metastases. *Scand J Gastroenterol*. 2013 **48**(10):1183-7.

20. Strippoli A, Rossi S, Martini M et al. ERCC1 expression affects outcome in metastatic pancreatic carcinoma treated with FOLFIRINOX: A single institution analysis. *Oncotarget*. 2016 **7**(23): 35159–35168.

21. Oliveira-Cunha M, Newman WG, and Siriwardena AK. Epidermal Growth Factor Receptor in Pancreatic Cancer. *Cancers (Basel)*. 2011 **3**(2): 1513–1526.

22. Heestand GM, Schwaederle M, Gatalica Z. Topoisomerase expression and amplification in solid tumours: Analysis of 24,262 patients. *Eur J Cancer*. 2017 83: 80–87.

23. Aoyama T, Miyagi Y, Murakawa M, et al. Clinical implications of ribonucleotide reductase subunit M1 in patients with pancreatic cancer who undergo curative resection followed by adjuvant chemotherapy with gemcitabine. *Oncol Lett*. 2017 **13**(5): 3423–3430.

24. Holdbrook T, Danenberg KD, Satti S, et al. ERCC1 and RRM1 as predictors of survival and response in pancreatic ductal adenocarcinoma treated with gemcitabine-based chemotherapy. 2011.

25. Kojima T, Kyuno D, and Sawada N. Targeting claudin-4 in human pancreatic cancer. *Expert Opin Ther Targets*. 2012 **16**(9):881-7.

26. Hamano H, Kawa S, Horiuchi A, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med*. 2001 **344**(10):732-8.

27. Sangha Brar JS, Gupta S, Mohideen SMH, et al. The pancreatic and extrapancreatic manifestations of IgG4-related disease. *Diagn Interv Radiol*. 2018 **24**(2):83–88.

28. Gao N, Le Lay J, Qin W, et al. Foxa1 and Foxa2 Maintain the Metabolic and Secretory Features of the Mature β-Cell. *Mol Endocrinol*. 2010 **24**(8):1594–1604.

29. Lee CS, Sund NJ, Behr R, et al. Foxa2 is required for the differentiation of pancreatic alpha-cells. *Dev Biol*. 2005 **278**(2):484-95.

30. Milan M, Balestrieri C Alfarano G, et al. FOXA2 controls the cis-regulatory networks of pancreatic cancer cells in a differentiation grade-specific manner. *EMBO J*. 2019 **38**(20).

31. Witt H, Beer S, Rosendahl J et al. Variants in CPA1 are strongly associated with early-onset chronic pancreatitis. *Nat Genet*. 2013 **45**(10):1216–1220.

32. Lasher D, Szabó A, Masamune A, et al. Protease-sensitive pancreatic lipase (PNLIP) variants are associated with early onset chronic pancreatitis. *Am J Gastroenterol*. 2019 **114**(6):974–983.

progress happens together

abcam

www.abcam.com