☑ Immunoassay Solutions for Biomarker Analysis

Immunoassays are invaluable research tools for measuring biological analytes across a range of drug discovery and development applications, including drug response testing, toxicity monitoring, and disease diagnosis. However, it can be time consuming and challenging to develop robust assays, especially in automated, high-throughput workflows. In this application note, we will describe Abcam's immunoassay solutions, from conjugation–ready matched antibody pairs against 1,600+ unique proteins for accelerating in-house immunoassay development to 1,500+ highly validated, ready-to-use SimpleStep ELISA® kits.

Matched antibody pairs: optimized for ELISAs

High-performance antibody pairs are the foundational building blocks for immunoassays. Each antibody in a matched pair targets a unique epitope on the same protein – one for capture, the other for detection. Matched antibody pairs can be adapted for various assay types, including sandwich ELISAs, TR-FRET, and bead-based platforms.

By virtue of recombinant manufacturing and rigorous QC, Abcam's antibodies (Fig. 1) are highly sensitive and specific – qualities paramount to the development of immunoassays. Our antibody pairs are selected from candidate clones and are required to undergo a methodical developmental workflow (Fig. 2), resulting in the production of pairs forming the basis of robust and reproducible assays (Fig. 3). Crucially, they are tested in plasma and serum to ensure specificity in complex samples. Furthermore, while the pairs are validated in a particular capture and detector orientation for sandwich ELISAs, the antibody orientation can be switched if found to be more suitable for a certain application.

Assay developers also need to conjugate a label of interest to the detector antibody. However, standard monoclonal antibodies are typically supplied with carrier proteins and other additives that can hinder effective conjugation. To enable the additional customization required for immunoassay development, all our antibody pairs are provided in a carrier-free format (BSA-, azide-, and glycerol-free).

Identifying high-performing matched antibody pairs is a lengthy and challenging process. Abcam's portfolio of 1,600+ matched antibody pairs is an unrivaled collection of recombinant monoclonals, providing assay developers a range of options against targets across oncology, neuroscience, immunology, metabolism, and other research areas.

Our recombinant antibodies: consistent, sensitive, and specific

Recombinant monoclonal antibodies are manufactured by cloning antibody-coding genes into expression vectors, enabling controlled antibody production that can be readily scaled for guaranteed long term supply with batch-to-batch consistency. On average, Abcam's high affinity recombinant antibodies have K_D values in the picomolar range. All antibodies undergo a battery of application-specific testing. Importantly, Abcam is at the vanguard of utilizing KO cell lines in validation workflows to determine antibody specificity at scale.

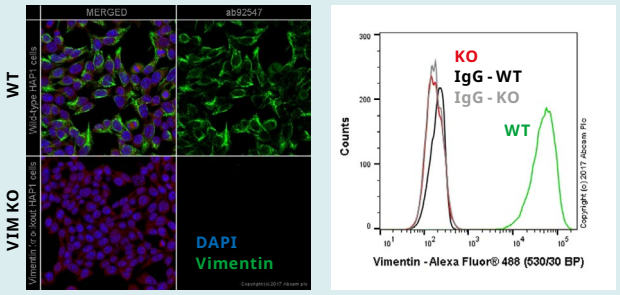


Figure 1. Recombinant Anti-Vimentin antibody [EPR3776] (ab92547) characterization data. To confirm specificity, ICC/IF and Flow Cytometry experiments were performed with a Vimentin knock-out cell line.

1. Screen multiple clones for best antibody pair candidates.
2. Antibody pairs tested for sensitivity, optimal orientation, and biological sample detection.
3. Best two pairs tested in both orientations with full standard curve and all required biologicals.
4. Spike/recovery, linearity, and cross-reactivity testing.

Figure 2. Abcam's matched antibody pairs development workflow.

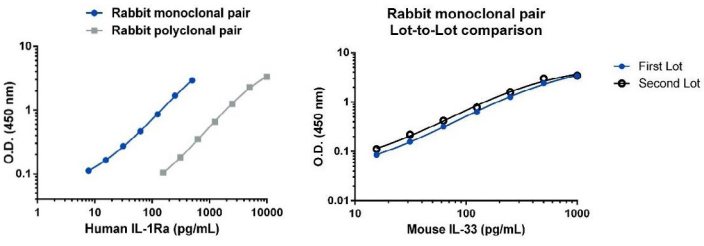


Figure 3. Immunoassays utilizing recombinant monoclonal antibodies exhibit high sensitivity and lot-to-lot consistency.

progress happens together abcam

☑ Tools for assay development

Abcam has developed a suite of complementary reagents to help researchers accelerate development of their immunoassays. These include Lightning-Link® conjugation kits for creating antibody-label combinations and knock-out cell lines and lysates for use as negative controls. As is the case with our recombinant antibodies and matched antibody pairs, these products undergo rigorous manufacturing processes and are tested extensively prior to publication.

Lightning-Link® conjugation kits

An important step in building an immunoassay is the labeling of the detector antibody. While any conjugation tool can be used, Abcam's Lighting-Link® kits have been configured for use with over 50 labels and can be readily utilized to create an antibody-label combination of choice. These kits are compatible with our entire range of carrier-free matched antibody pairs.

With a simple two step protocol, Lightning-Link® conjugation kits make it easy to generate conjugated detector antibodies with batch-to-batch consistency (Fig. 4). As Lightning-Link kits do not require a post-conjugation purification step, antibody is not lost throughout the process. These kits can conjugate up to 100 mg of antibody at a time, and thus remain effective as experimental workflows scale.

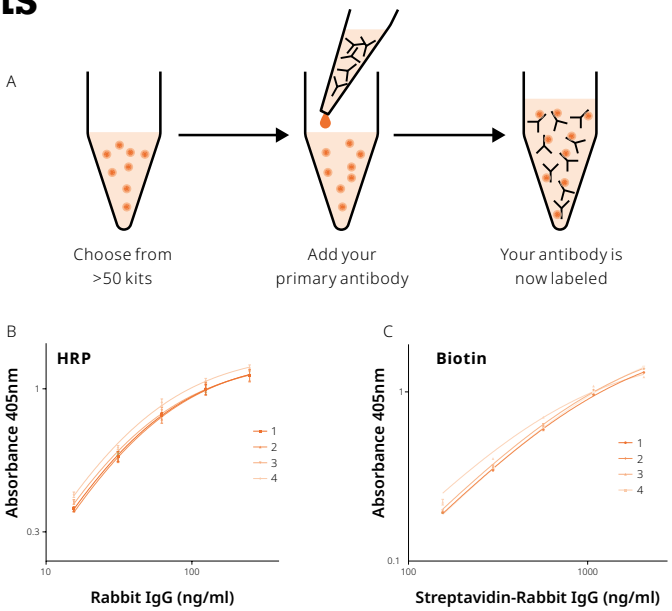


Figure 4. Lightning-Link® kits have a simple protocol that yields consistent results. A) Diagram illustrating the protocol. B,C) Batch-to-batch consistency of HRP (B) and Biotin (C) kits tested in sandwich ELISA. Each 100 µg vial was conjugated to 100 µg of Goat anti Rabbit IgG overnight (B) or 100 µg of HRP for 15 minutes (C).

CRISPR knock-out cell lines and lysates

Knock-out (KO) cell lines are valuable tools for determining the specificity of the antibody pair (Fig. 5) and can also act as negative controls during experiments.

Additionally, KOs can be particularly useful when the target protein is present in low abundance as in these cases it can be difficult to reliably differentiate between true signal and background noise at the lower limits of detection.

However, many assay developers prefer the cell lysate form as this eliminates the need for tissue culture. To accommodate this need, we also offer a collection of 2,000+ assay-ready cell lysates that were prepared from our extensive portfolio of KO cell lines.

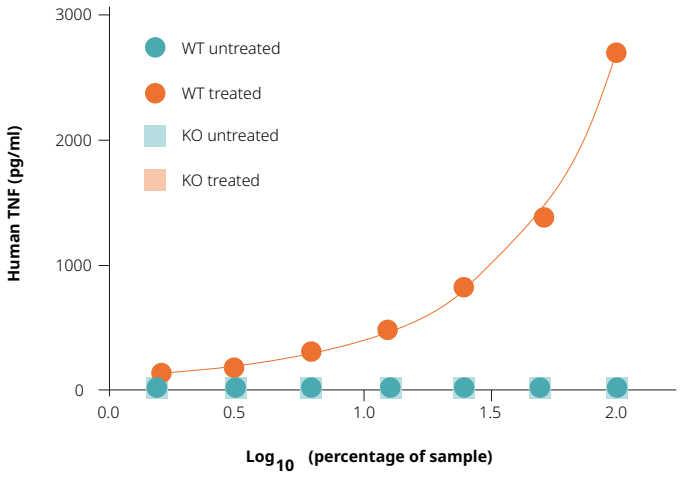


Figure 5. Human TNF-α ELISA kit (ab181421) characterization data of cell culture supernatant. WT THP-1 cells and TNF-α KO THP-1 cells (ab273761) were assessed in duplicate (n=2). Cells were treated with 100 ng/ml LPS for 16 h to induce expression of TNF-α.

SimpleStep ELISA® kits

Rather than building an ELISA assay from scratch, using a validated kit can save valuable time. SimpleStep ELISA® kits are sandwich immunoassays that have been developed to be as simple and effective as possible for use in complex biological samples. Unlike traditional sandwich ELISAs, which have run times of 3 to 6 hours, SimpleStep ELISA® kits generate data in only 90 minutes with a simple, one-wash step protocol. Because the assays are developed using our recombinant monoclonal antibody pairs, they exhibit exceptional sensitivity and consistency (Fig. 6). We currently offer a portfolio of over 1,000 kits, which represents the broadest target coverage on the market. Indeed, many of Abcam's kits were launched as first-to-market products, providing researchers with ready-to-use immunoassays to analyze novel biomarkers.

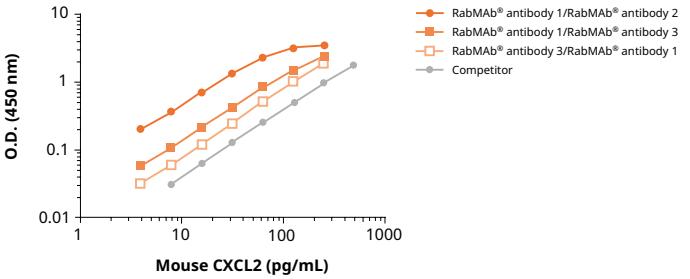


Figure 6. Antibodies are selected for optimal performance in ELISA. In this example, three of Abcam's antibody pairs against the mouse chemokine CXCL2 show superior sensitivity to a competitor's standard ELISA. High sensitivity is an important factor for this chemokine since CXCL2 levels are typically less than 20 pg in undiluted mouse serum.

How abcam is innovating on sandwich ELISAs

A conventional sandwich ELISA uses a pair of antibodies (capture and detector) that bind to two distinct epitopes on the target antigen. Depending on the protocol, multiple incubation and wash steps are typically required.

In a SimpleStep ELISA® kit, the sample along with the capture and detector pair are incubated together, enabling the antibody-antigen complex to form in a single step (Fig. 7). Since the capture antibody contains an immunoaffinity tag that binds to the immobilization antibody pre-coated to the microplate well, the sandwich complex is anchored to the plate. Prior to addition of the detection reagent, unbound sample and antibody is removed with one wash step.

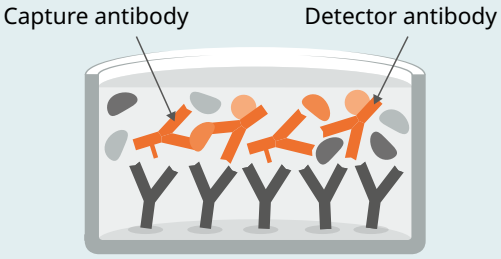
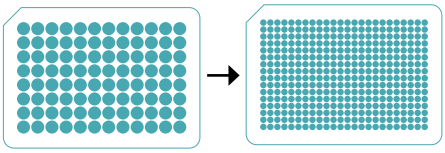


Figure 7. In a SimpleStep ELISA®, an analyte/antibody complex is formed in solution and binds to the surface via an affinity tag.

384-well format: scaling up for high-throughput applications

Many scientists are now running ELISA assays on automation platforms that analyze thousands of samples. To address this increased throughput, we have further developed the SimpleStep ELISA® into a 384-well format, compatible with automated workflows. The 384-well assays are based on the same principles (Fig. 8) that underlie the 90 minute 96-well assays, which allows them to maintain sensitivity and reproducibility (Fig. 9, 10) while also allowing them to have increased throughput and reduced sample requirements.



96-well
50 µl sample volume
90-minute assay

384-well
12.5 µl sample volume
90-minute assay

Figure 8. Miniaturizing the 96-well assay into an automation-friendly 384-well version.

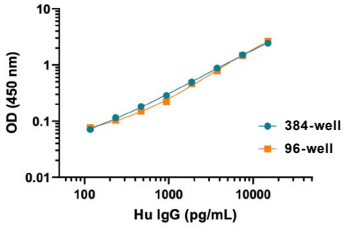


Figure 9. Example of Human IgG standard curve.

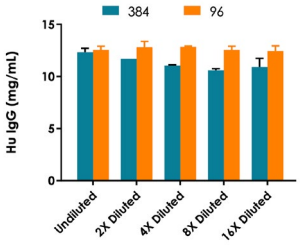


Figure 10. Human IgG was measured in biological samples in a 2-fold dilution series.

☑ Immunoassay Solutions Quick Guide

Matched antibody pairs

– 1,600+ antibody pairs

– Recombinant monoclonal technology

– Validated in sandwich ELISA

– Includes capture / detector pair

– Carrier-free, conjugation-ready

Lightning-Link® conjugation kits

– 50+ labels

– Simple, three step protocol

– 30 seconds of hands-on time

– Works with 10 µg-100 mg of antibody

Knock-out cell lines and lysates

– 5,000+ cell lines, 2,000+ lysates

– CRISPR multi-gRNA approach

– Individually cloned

– Includes wild-type controls

SimpleStep ELISA® kits

– 1,000+ unique targets

– Simple, 90-minute, single-wash protocol

– 10x pack and 384-well format available

– Validated on biological samples

– Calibrated against international standards

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