

A grayscale photograph of a laboratory setting. In the foreground, there are several glass bottles and test tubes on a metal rack. A hand in a white glove is visible in the upper left, holding a pipette. The background is slightly blurred, showing more laboratory equipment and a person in a white lab coat.

Custom Services of ABclonal



● Research-use antibody development

- WB-guaranteed polyclonal antibody
- Application validated monoclonal antibody
- Neutralizing/blocking antibody
- Mutation-specific antibody
- Modification-specific antibody
- Small molecular antibody

● Custom antibody formulation and conjugation

● Antibody engineering

● IVD antibody development

● ELISA antibody pair testing

● ELISA kit development

● Antibody-based immunoassay development

● Protein expression service

- Prokaryotic expression system
- Insect expression system
- Mammalian expression system

● Peptide services

- Peptide synthesis
- Peptide library

■ CORE SERVICES

Outsource your antibody production / Save time to focus on your research

Leveraging our expertise in antibody production, we provide you with customized services for application-specific polyclonal and monoclonal antibodies. A full set of antibody related services are available in ABclonal, including antigen design, immunogen preparation, antibody development, antibody purification, modification, validation and immunoassay development.

// Service Highlights

Free Antigen Design

Proper selection and design of a suitable antigen is the most critical step before getting a useful antibody.

High-quality Antigen

According to project design, high-quality purified proteins or peptides will be used as antigens.

Broad Range of Species

ABclonal scientists have comprehensive experiences of antibody development in broad range of species.

High Specificity

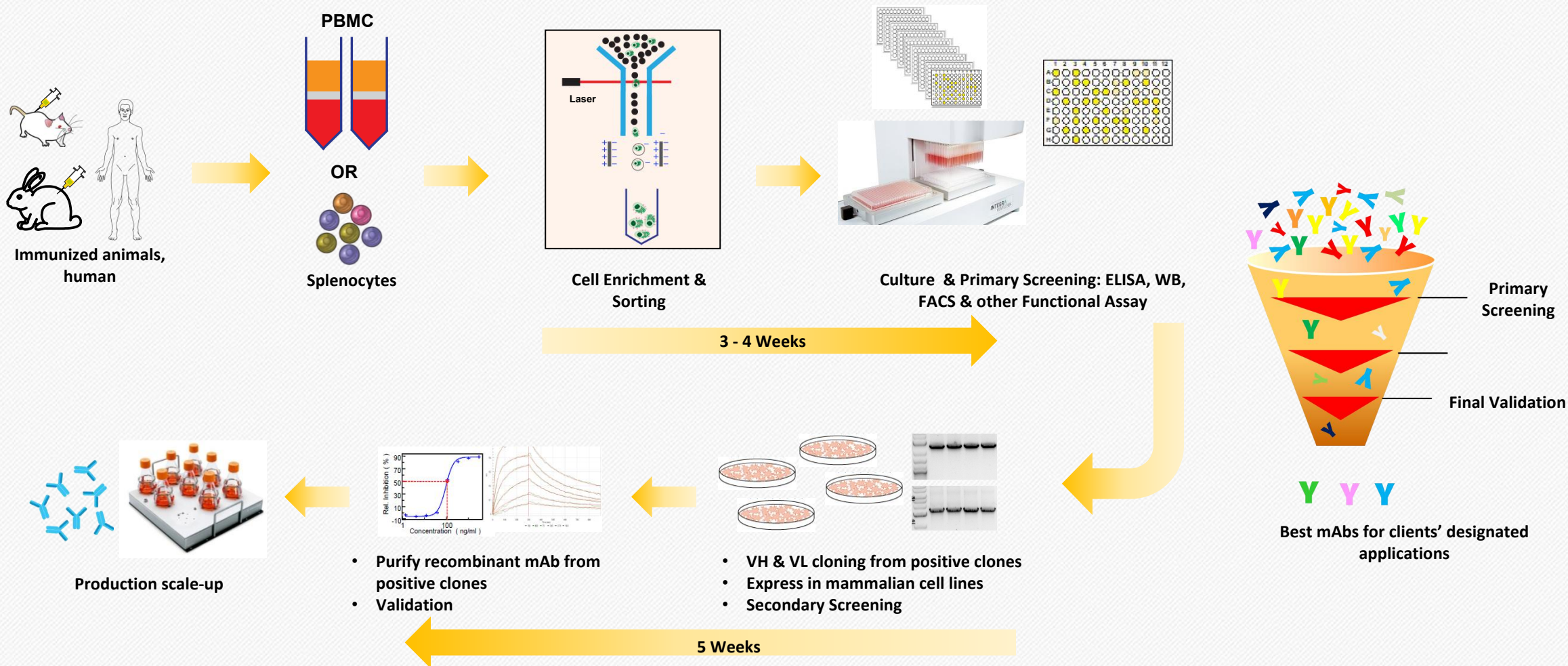
Advanced antibody development technology, stringent screening and validation, high standard purification system, will ensure high specificity of antibody that we developed for you.

Guaranteed Results

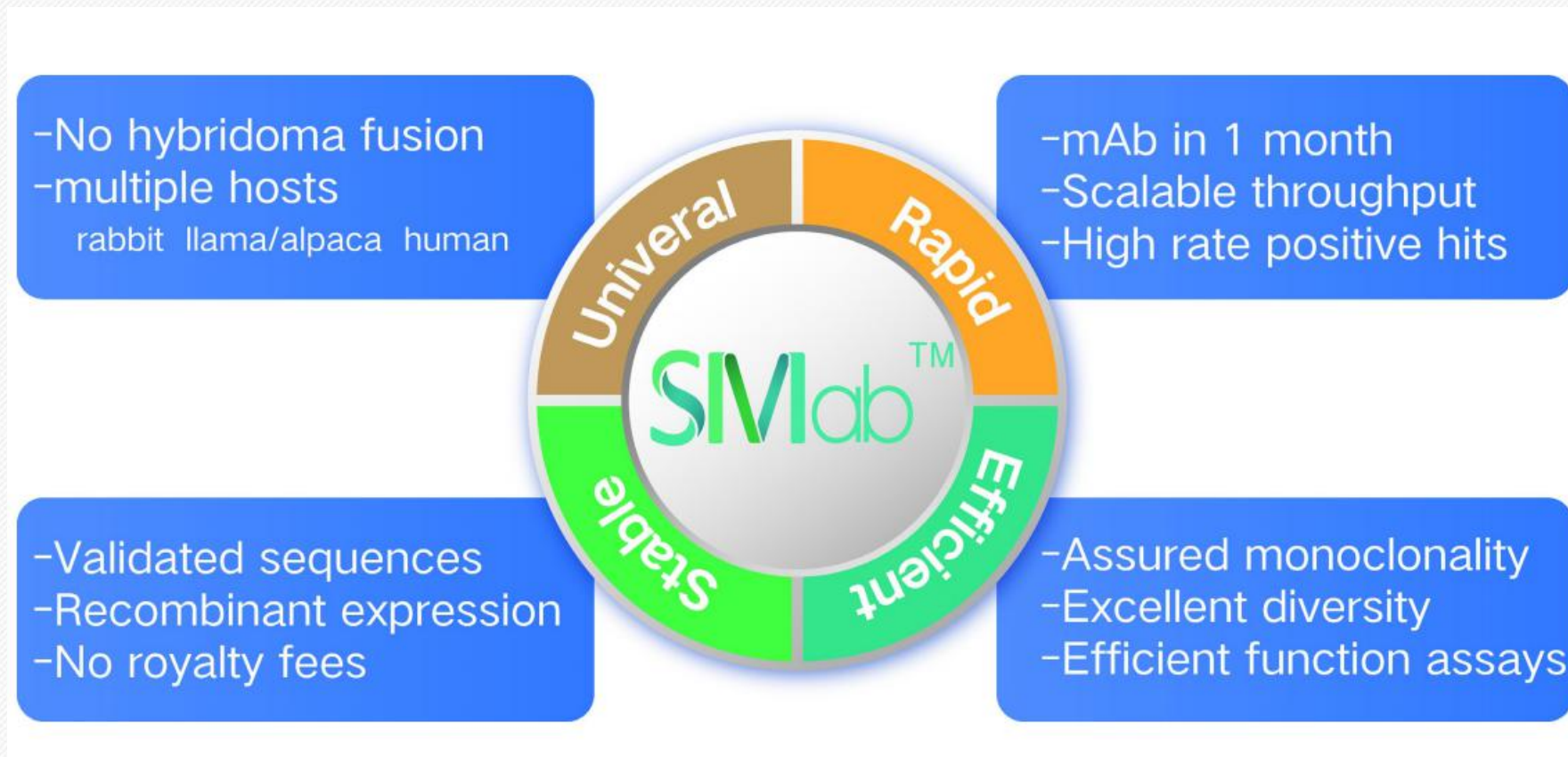
Our in-house validation will ensure good performance in requested applications before you get the antibody.

Introduction of SmAb™ Platform

SmAb: Single cell-based, **S**peedy platform delivers **S**uperior **mAb** to customers.

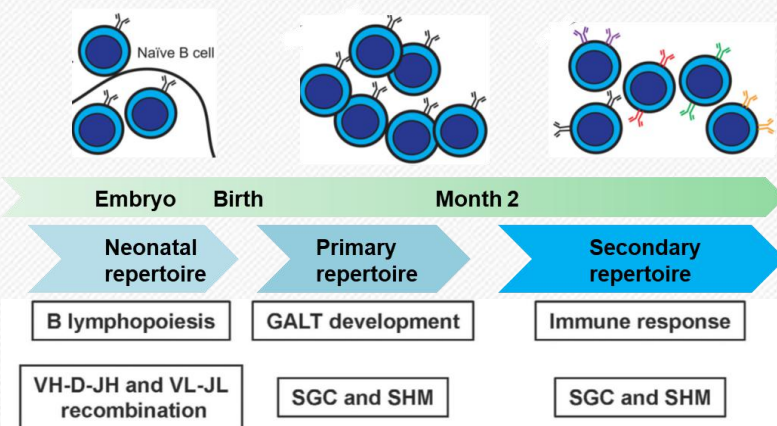


SmAb: Single cell-based, Speedy platform delivers Superior mAb to you



Advantage of Rabbit Monoclonal Antibody

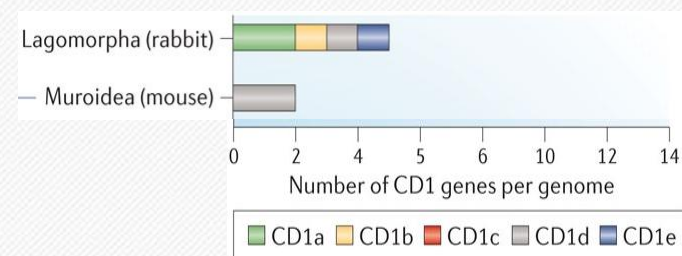
Unique Immune System



Highly diverse B repertoire

- gene conversion rate
- rich allotypes
- diverse VH
- longer CDR3 in light chain

More categories of CD1



- ✓ **High Affinity:** ~ 10 times higher than mouse mAbs
- ✓ **High Specificity:** Ideal for IHC staining.
- ✓ **Great Diversity:** broad epitope recognition and conformational epitope recognition.

Excellent Presentation for Hapten Antigen. Ideal for:

- Post-translational modification site specific antibodies
- Anti Small Chemical Compound Antibodies
- Anti nucleotide (DNA/RNA) Antibodies
- Polysaccharides

Reference:

1. Kennedy DE, Witte PL, Knight KL. **Bone marrow fat and the decline of B lymphopoiesis in rabbits.** *Dev Comp Immunol.* 2016 May; 58: 30–39.
2. Justus Weber, Haiyong Peng and Christoph Rader. **From rabbit antibody repertoires to rabbit monoclonal antibodies.** *Experimental & Molecular Medicine* (2017) 49

Comparison of Antibody Development Technologies

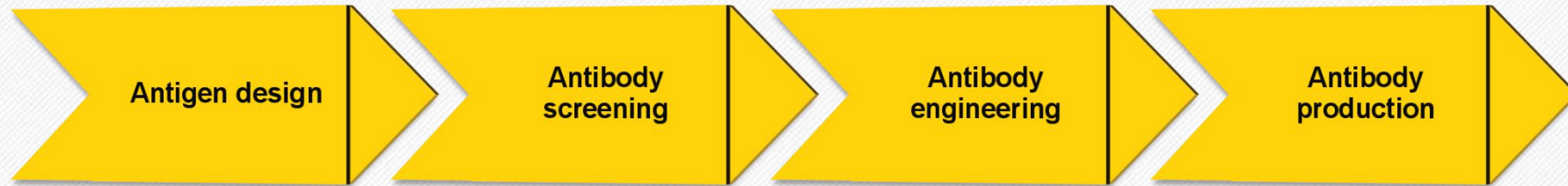
	Hybridoma	Phage/Yeast Display	Single B Cell sorting/cloning
Start Material	Spleen	Spleen or PBMC	Spleen or PBMC
Screening Time	> 12 weeks	6 weeks	3 weeks
Efficiency	Low	Medium	High
Antibody heavy & light chain pairing	Natural	Artificial	Natural
Final Delivery	Hybridoma	Plasmids carrying mAb sequences	Plasmids carrying mAb sequences
Stability of Final Products	Low	High	High
Sequences of mAb	Unknown	Known	Known
mAb Diversity	Medium	Low	High
Host Species	Mouse, Rat, Rabbit	Mouse, Rat, Rabbit, Llama, Human	Mouse, Rat, Rabbit, Monkey, Human

Comparison of Single B cell Based Antibody Development Platform



Vendor	Recombinant Ab development platform	Major innovation	Pros and Cons
Abcam	RabmAb® Technology	<p>Hybridoma RabmAb: Rabbit antibody-secreting B cell fusion with proprietary partner cells (240E-W2, US patent 742987)</p> <p>NGS based RabmAb® Technology: Direct B cell IgG gene library generation and NGS, large bioinformatics</p> <p>B cell cloning RabmAb® Technology: Direct cloning of antibody genes from single B cells by RT-PCR and PCRs and the in vitro recombinant expression verification</p>	<p>Pros: Successful case experience</p> <p>Cons: delayed functional assay validation, high cost</p>
SinoBiological	Single B Cell Antibody Discovery	Direct cloning of antibody genes from single B cells by RT-PCR and PCRs and the in vitro recombinant expression verification	<p>Pros: No innovation</p> <p>Cons: delayed functional assay validation, high cost, time consuming</p>
Genescript	MonoRab™	Secreting B cell fusion with proprietary partner cells for single B cell expansion	<p>Pros: relative lower cost, early screening by functional assay, high efficiency in positive B cell clone identification</p> <p>Cons: not natural B cell expansion</p>
ABclonal	SmAb® Technology	Direct B cell in-vitro culture and expansion. In this technology, an optimized rabbit culture medium is used to culture the individual rabbit B lymphocytes obtained by sorting. The B cells are stimulated to proliferate in vitro and secrete a sufficient amount of antibody IgG for primary screening.	<p>Pros: relative lower cost, early screening by functional assay, high efficiency in positive B cell clone identification</p> <p>Cons: natural B cell</p>

One-stop Antibody Development Services



Antigen design

- Cell Immunization
- VLP Immunization
- mRNA immunization

Antigen production

- mammalian system

Antibody screening

SmAb® platform

- Rabbit
- Llama
- Porcine
- Monkey
- Human**

Antibody engineering

- Fab cloning
- Class switch
- ScFv construct
- Ab sequencing
- Ab humanization

Antibody production

- Transient expression
- Stable cell line



Target Customer Segments



Applications:

- ✓ High Sensitive In Vitro Diagnostic Immunoassay Development



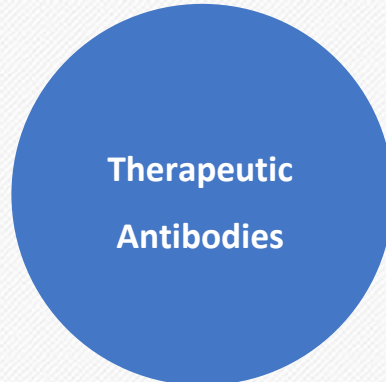
Applications:

- ✓ Pharmacokinetic (PK) Studies
- ✓ Immunogenicity Analysis
- ✓ Vaccine development



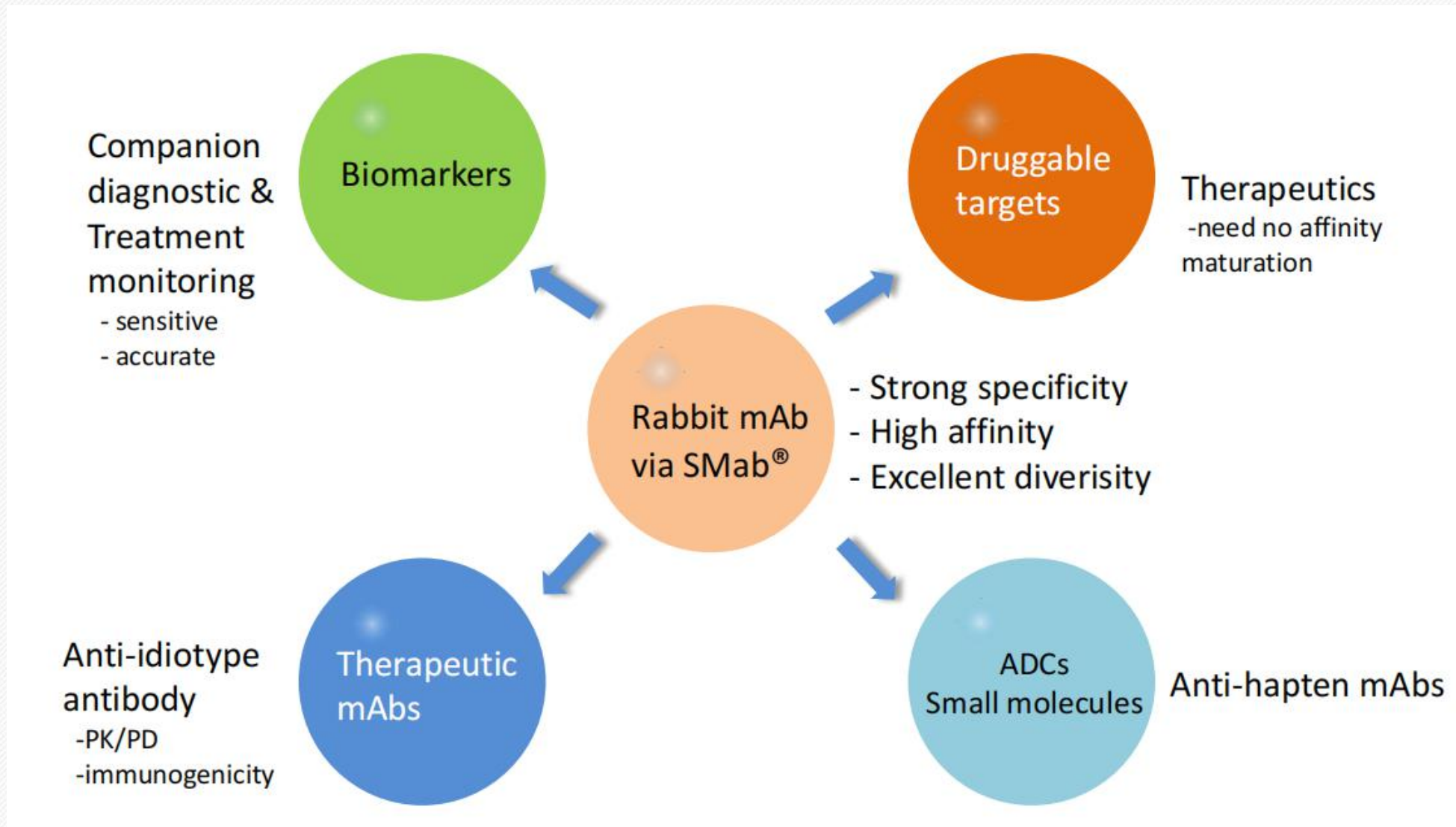
Especially ideal For:

- ✓ Hapten
- ✓ High conserved epitope
- ✓ Post-translational modification
- ✓ Poor immunogenicity in mouse



Clinical Name	Target	FDA Approve clinical number	Progress	Company
Brolucizumab	VEGF	NCT02307682, NCT02434328	FDA approved	Novartis
Eptinezumab	CGRP	NCT04152083, NCT02974153	FDA approved	Alder/Lundbeck
Clazakizumab	IL-6	NCT03744910	III	Alder BioPharmaceuticals
BD0801	VEGF	NCT02453464	II	Simcere/Apexigen
SSS07	TNFalpha	NCT02460393	Ib	3SBio/Apexigen
APX005M	CD40	NCT03123783	II	Apexigen
YYB101	HGF	NCT02499224	I	YooYoung Pharmaceutical

ABclonal Recombinant Rabbit mAbs for Biopharma



Service workflow



STEP 1
Pre-sales
Communication

QUOTE

First Name * **Last Name *** **Work Email ***

Phone Number * **Institution Name ***

How did you know about ABclonal *

Antibody Quote Item #1

If a UniProt ID or NCBI Accession Number is not available, please provide the nucleotide sequence (From ATG to termination codon) and/or the amino acid sequence

Should the antibody target Post-Translational Modification (PTM) sites or residues?
 Yes No

Should the antibody target mutated sites or residues?
 Yes No

For non-protein targets, please describe the target here:

Type of antibody

- Rabbit monoclonal
- Rabbit polyclonal
- Mouse monoclonal
- Alternate species

Desired antibody application(s)

- WB
- IHC/ICC/IF
- IP/Co-IP
- ELISA-Pair
- ELISA
- Flow cytometry
- ChIP
- Neutralizing antibody
- Blocking antibody

Cross-reactivity with other proteins

Cross-reactivity with other species

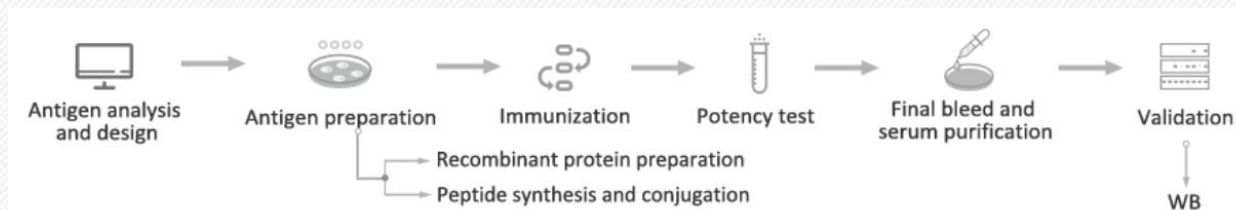
Other requirements



STEP 9
Product
delivery

Timeline of Antibody Development Services

// Polyclonal Antibody Production Process



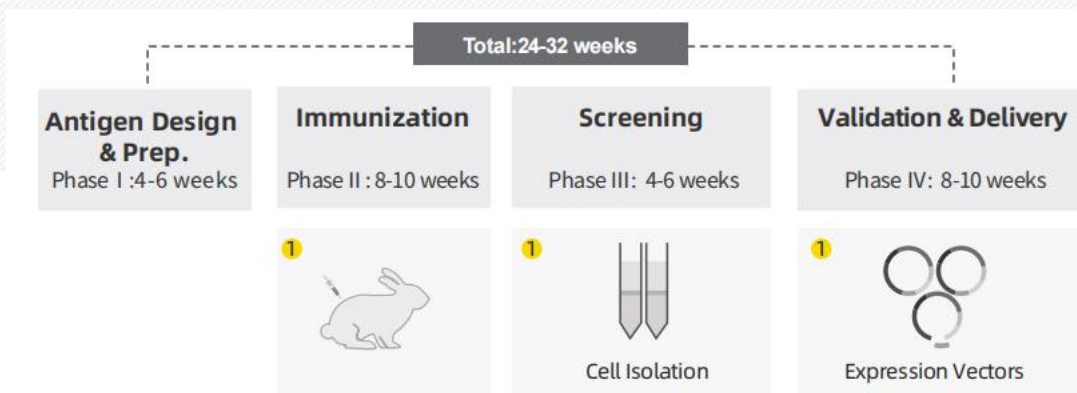
Timeline:

- If the customer provides the antigen: 10 weeks;
- If ABclonal prepares the antigen: 14 weeks (protein antigen) 13 weeks (peptide antigen);

Deliverables:

- ✓ Final Report
- ✓ 2 mg of purified antibodies
- ✓ Immuogens: protein (0.1mg), peptides (2mg)

// Rabbit Monoclonal Antibody Production Process



Deliverables during service process :

- ✓ Anti-serum for each rabbit before- and after-immunization
- ✓ Single B cell supernatant (ELISA positive)
- ✓ LEM (Linear expression model) supernatant (ELISA positive)

Final Deliverables:

- ✓ Final Report
- ✓ 100 µg of each recombinant rabbit monoclonal antibody (ELISA positive)
- ✓ Antibody expression plasmid of selected positive clone
- ✓ Sequence of antibody genes of selected positive clone

FAQ of Antibody Development Services



- Q1: What information does the customer need to provide for custom antibody development?
- A1: ABclonal will provide standard service information collection form for customer, including the expected applications of the customized antibody, the customer's specified requirements for the antibody, antibody type (monoclonal or polyclonal antibody), species of samples, gene ID, gene sequence (protein sequence), etc.
- Q2: What analysis does the antigen design report include?
- A2: Protein specificity analysis, protein conservation analysis, signal peptide prediction, hydrophilicity, immunogenicity and epitope exposure, secondary structure prediction, tertiary structure analysis, antigenicity analysis-linear B cell epitopes, antigen type and antigen region selection suggestions, etc.
- Q3: What are the characteristics of different types of antigens?
- A3: Recombinant proteins generally have multiple antigenic determinants, some of which are sequence determinants, and some are structural determinants. The polyclonal antibody obtained by immunizing animals with recombinant protein is a mixture of antibodies against each antigenic determinant, which can be used to detect natural structure or denatured target protein. Recombinant protein antigens are often more immunogenic and can stimulate animals to have a strong immune response. In order to improve the possibility of target protein expression and the convenience of purification, we often select to express a small fragment of the target protein, such as a specific domain.
- Peptides are selected epitopes predicted by various software, and antibodies with strong specificity and low cross-reactivity can be prepared through epitope selection. It is economical and fast to use synthetic peptides as antigens, but there is a risk of weak or non-reactive peptides due to the improper selection. Therefore, when preparing antibodies from peptide antigens, two or three different peptides are often alternative to ensure the success rate of antibody selection.
- Q4: What is the method of antibody purification?
- A4: Antigen affinity purification is used in preparation of polyclonal antibodies, and Protein A/G purification is used in preparation of monoclonal antibodies.

FAQ of Antibody Development Services



- Q5: In the whole process, in which steps do we need the customer to participate?
- A5: For polyclonal antibody, we just need the customer to test the final delivered antibody and give feedback.
- For monoclonal antibody, the tests that will need the customer including
 - ✓ Anti-serum for each rabbit before- and after-immunization
 - ✓ Single B cell supernatant (ELISA positive)
 - ✓ LEM (Linear expression model) supernatant (ELISA positive)
 - ✓ Final delivered antibody
- Q6: What kind of after-sales technical support can ABclonal provide to the customer?
- A6: If the antibody test results are not satisfactory, we will provide the technical support form for troubleshooting, we suggest the customer repeating the experiment based on our first-round suggestions. If you still have a problem with this antibody, we will recall the antibody back to perform in-house testing and provide sound solutions further.

Successful cases

Case 1: High Specific Rabbit anti-human IgG Fc Monoclonal Antibody Development

Background: During preclinical studies, there is a great need to develop monoclonal antibodies (mAbs) that are specific to human immunoglobulin (IgG), without binding to monkey IgG.

Immunize Two Rabbits with Fc Fragment of humanized mAb

Stable monoclonal antibody clones with specific binding to human IgG, but not to monkey IgG.

Total Sorted Clones	1920
Human IgG positive/Cy5 IgG Negative	58

Outcome: 58 clones for final selection.

Case 2: High Affinity Rabbit Monoclonal Antibodies

Rabbit anti-hIL2 Monoclonal Antibodies

Hypersensitive High Specificity

Affinity Measured by Biacore 3000

Clone	hIL2/12	hIL2/16	hIL2/18
Clone 285	2.0E+07	2.0E+07	2.0E+07
Clone 304	2.0E+07	2.0E+07	2.0E+07
Clone 314	2.0E+07	2.0E+07	2.0E+07

Rabbit anti-hIL6 Monoclonal Antibodies

Hypersensitive High Specificity

Affinity Measured by Biacore 3000

Clone	hIL6/12	hIL6/16	hIL6/18
Clone 307	1.5E+07	1.5E+07	1.5E+07
Clone 311	1.5E+07	1.5E+07	1.5E+07

Case 3.1: Great Diversity of Rabbit Monoclonal Antibodies

Requirements:
 1. A pair of rabbit anti-hIL13 mAbs
 2. Bind to different epitope with bi-specific antibody provided by client

1920 single B cell clones
 236 positive clones (12.2%)
 Randomly pick 4 best clones for cloning

Final Sandwich ELISA Validation

2C4 (Capture) — hIL13 (Detection)

Outcome: 4 clones selected for cloning.

Case 4: Anti-Hapten Rabbit Monoclonal Antibodies

Rabbit Anti-Histopregnane (HAP) Monoclonal Antibody

A hapten used in vaccine preparation

After two months immunization with HAP conjugated BSA

1920 single B cell clones
 positive clones (12.2%)

Rabbit Anti-Mucinase Specific Antibody

Immunogen: K12 conjugated BSA

After three months immunization with immunogen

1920 single B cell clones
 126 positive clones
 76 clones no cross reactivity to dN, dE, dC
 Picked 4 clones for final cloning

Case 5: Rabbit Monoclonal Antibody For Post-translational Modification Site

Rabbit Anti-pT40-PTEN Specific Antibody

Inhibition of Nuclear PTEN Tyrosine Phosphorylation Enhances Glutathione Sensitivity Through Altered DNA Repair

Sort with phosphorylated peptide

1920 Single B cell clones
 Randomly pick 11 clones for next step

Outcome: 11 clones selected for next step.

Case 6: Anti-Idiotypic (Anti-ID) Rabbit Monoclonal Antibodies Development

Anti-ID Rabbit Monoclonal Antibodies Development For Traditional Therapeutic Antibody

Anti-Idiotypic Antibody Development

1920 Single B cell clones
 302 Positive Clones (rate 28%)
 168 Blocking anti-id clones (Type 1)
 222 non-blocking anti-id clones (Type 2)

Randomly Pick 5 clones (3 Type 1 & 2 Type 2)

Outcome: 5 clones selected for final validation.

Case 7: Develop Rabbit Monoclonal Antibody with Cell/Virus as Antigen

Use Stable CHO cell line as Immunogen

OC of immunization

1920 Single B cell clones
 169 positive clones
 69 FACS clones
 18 FACS clones
 23 Clones for neutralization

Use Virus Like Particles (VLP) as Immunogen

After 60 days immunization with Variant 3 VLP

1920 Single B cell clones
 886 positive clones (rate 46%)
 16 neutral 1 specific clones
 29 neutral clones (rate 1.5%)
 87 neutral clones (rate 4.5%)
 19 neutral clones (rate 1%)
 19 neutral clones (rate 1%)

Case 8: Therapeutic Rabbit Monoclonal Antibodies Development

Rabbit Anti-hCD40 Antagonist Antibody Discovery

hCD40 (hCD40) signal pathway

In Vitro Reporter Assay

PMSC Stimulation Assay

1920 Single B cell clones
 417 positive clones (21.7%)
 24 neutral clones (1.2%)
 24 neutral clones (1.2%)

Case 9: SMab™ Platform for Nanobody Development

Immunize Alpaca with 267D nCDV Splice S1 Protein

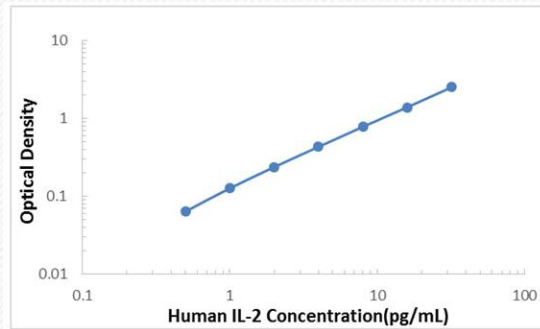
1920 Single B cell clones
 30 positive clones (1.5%)
 26 neutral clones

Pseudo-Virus Neutralization Assay

Case 1: High Affinity Rabbit Monoclonal Antibodies

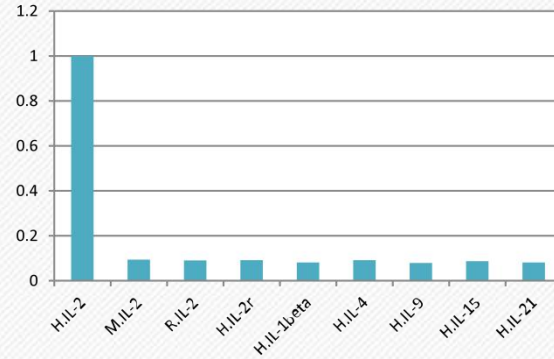
Rabbit anti-hIL2 Monoclonal Antibodies

Hypersensitive



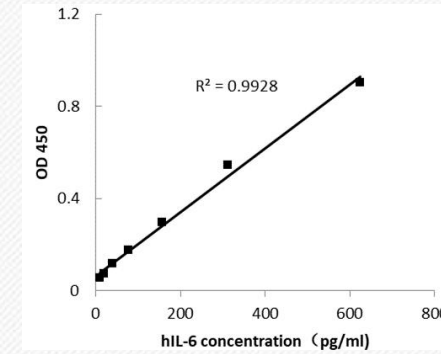
Sensitivity: ~ 0.7pg/ml

High Specificity

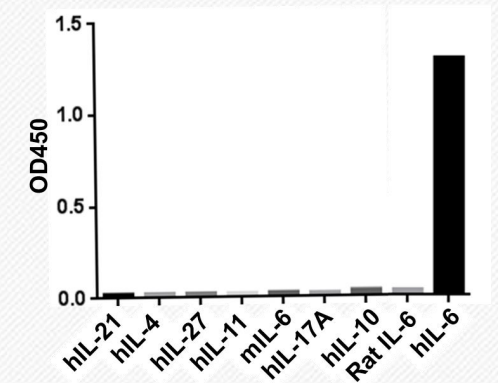


Rabbit anti-hIL6 Monoclonal Antibodies

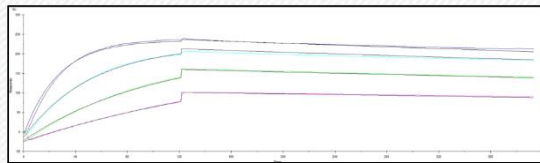
Hypersensitive



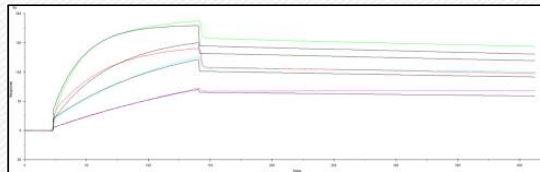
High Specificity



Affinity Measured by Biacore 3000



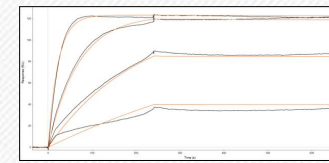
Clone 2B5



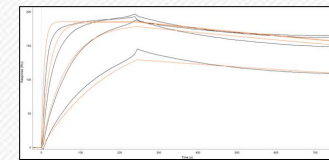
Clone 8G8

Clone number	k _{off} (1/s)	k _{on} (1/Ms)	K _D (M)
2H5	5.25 X 10 ⁻⁴	5.83 X 10 ⁵	8.99 X 10 ⁻¹⁰
8G8	3.75 X 10 ⁻⁴	1.28 X 10 ⁶	2.93 X 10 ⁻¹⁰

Affinity Measured by Biacore 3000



Clone 3D7

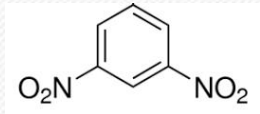


Clone 2F1

Clone number	K _{off} (1/s)	K _{on} (1/Ms)	K _D (M)
3D7	1.34 X 10 ⁻⁵	3.57 X 10 ⁶	3.75 X 10 ⁻¹²
2F1	3.28 X 10 ⁻⁴	4.19 X 10 ⁵	7.80 X 10 ⁻¹⁰

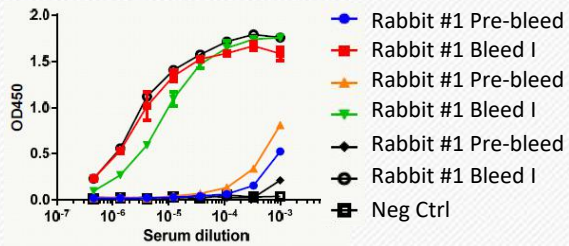
Case 2: Anti-Hapten Rabbit Monoclonal Antibodies

Rabbit Anti-Dinitrophenyl (DNP) Monoclonal Antibody

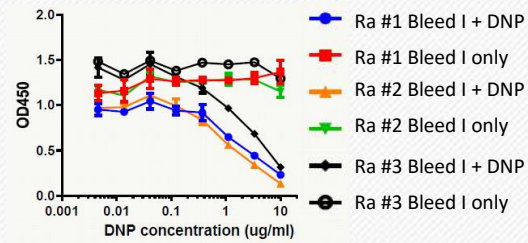


A hapten used in vaccine preparation

After One Month Immunization with KLH conjugated DNP

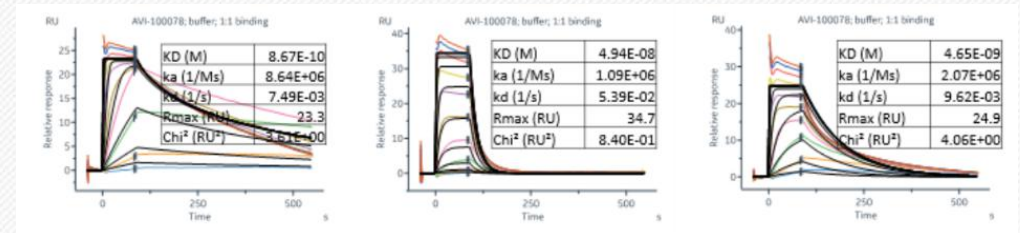


Regular ELISA with BSA conjugated DNP



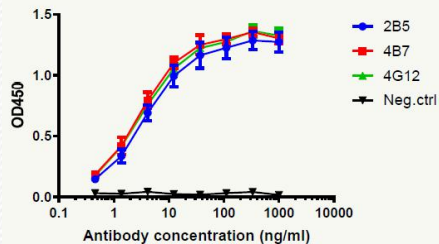
Competition ELISA with BSA conjugated DNP

Affinity Measured by Biacore 3000

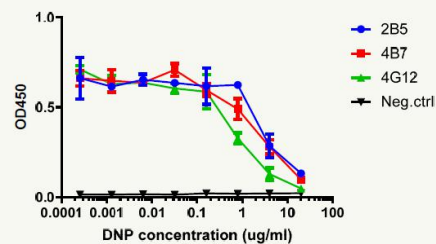


1920 single B cell clones

234 positive clones (12.2%)



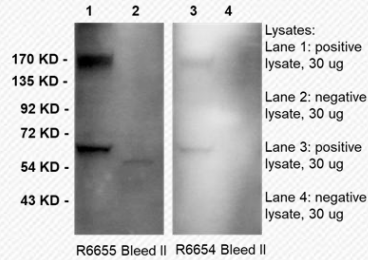
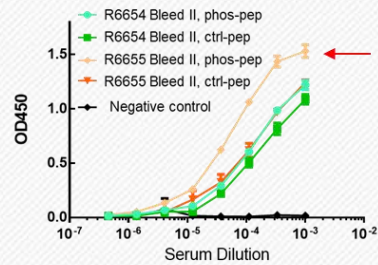
Regular ELISA with BSA conjugated DNP



Competition ELISA with BSA conjugated DNP

Case 3: Rabbit mAb For Post-translational Modification Site

Prescreening QC

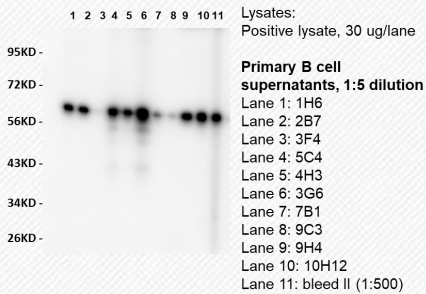


Sort with phosphorylated peptide

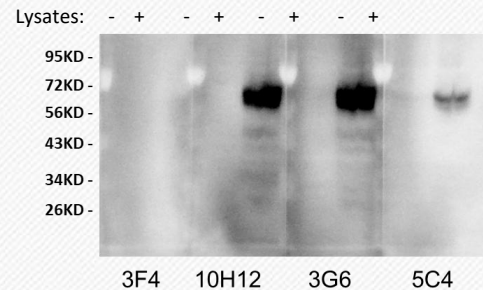
1536 Single B cell clones

117 Phosphorylated peptide positive/non-phosphorylated peptide negative clones

Randomly pick 11 clones for mini-WB



WB with final purified 4 clones



mAb dilution: 1 ug/ml each clone

-: negative lysate: non-phosphorylated target protein

+: positive lysate, phosphorylated target protein

Rabbit Anti-pY240-PTEN Specific Antibody

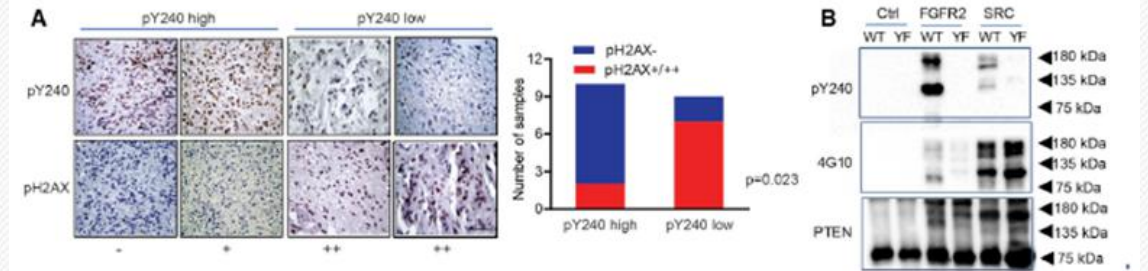
Cancer Cell Article

CellPress

Inhibition of Nuclear PTEN Tyrosine Phosphorylation Enhances Glioma Radiation Sensitivity through Attenuated DNA Repair

Jianhui Ma,¹ Jorge A. Benitez,¹ Jie Li,² Shunichiro Miki,¹ Claudio Ponte de Albuquerque,¹ Thais Galatro,³ Laura Orellana,^{4,5,6} Ciro Zanca,¹ Rachel Reed,¹ Antonia Boyer,¹ Tomoyuki Koga,¹ Nissi M. Varki,⁷ Tim R. Fenton,⁸ Suelly Kazue Nagahashi Marie,^{3,9} Erik Lindahl,^{4,5,6} Timothy C. Gahman,^{1,14} Andrew K. Shiau,¹ Huilin Zhou,¹ John DeGroot,¹⁰ Erik P. Sulman,¹¹ Webster K. Cavenee,^{1,2,12} Richard D. Kolodner,^{1,2,13} Clark C. Chen,² and Frank B. Furnari^{1,2,7,15,*}

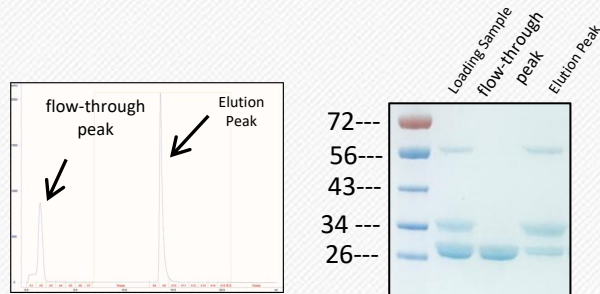
¹Ludwig Institute for Cancer Research, San Diego Branch, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0660, USA



Case 4: Anti-Idiotypic (Anti-ID) Rabbit mAb Development

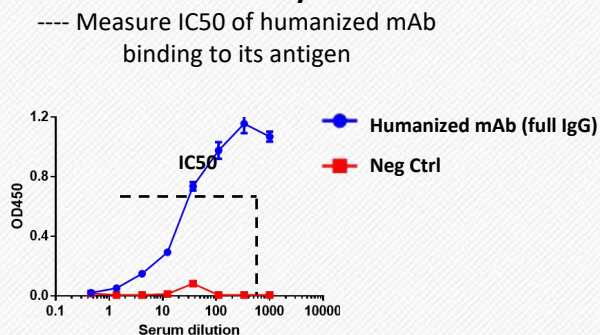
Anti-ID Rabbit Monoclonal Antibodies Development For Traditional Therapeutic Antibody

Fab Antigen Preparation

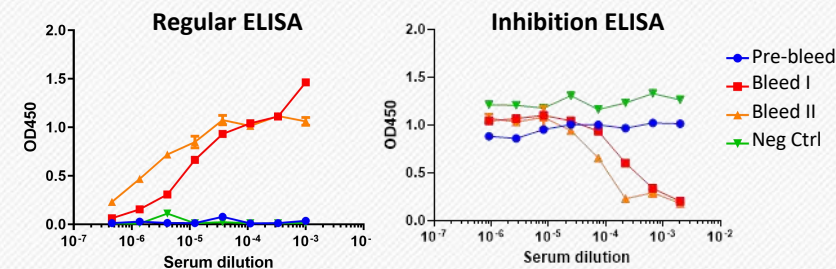


Column: mAbSelect Sure

Pilot Study



Validation of Immunized Serum

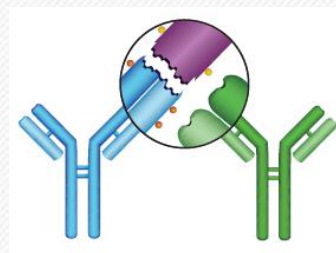
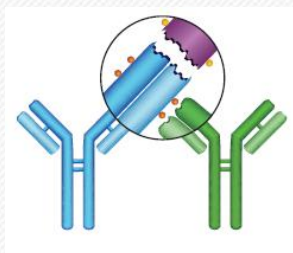
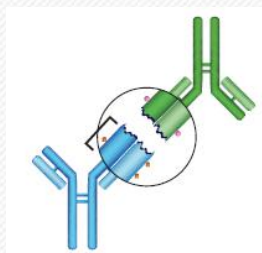


1920 Single B cell clones

382 Positive Clones (rate 20%)

160 **blocking** anti-ID clones (Type 1)

222 **non-blocking** anti-ID clones (Type 2)



✓ **Type I: blocking**

✓ Specific to paratype

✓ Blocking antibody-antigen binding

✓ Measure free therapeutic antibody in blood

✓ **Type II: non-blocking**

✓ No binding to paratype

✓ Not block antibody-antigen binding

✓ Measure total level of therapeutic antibody in blood

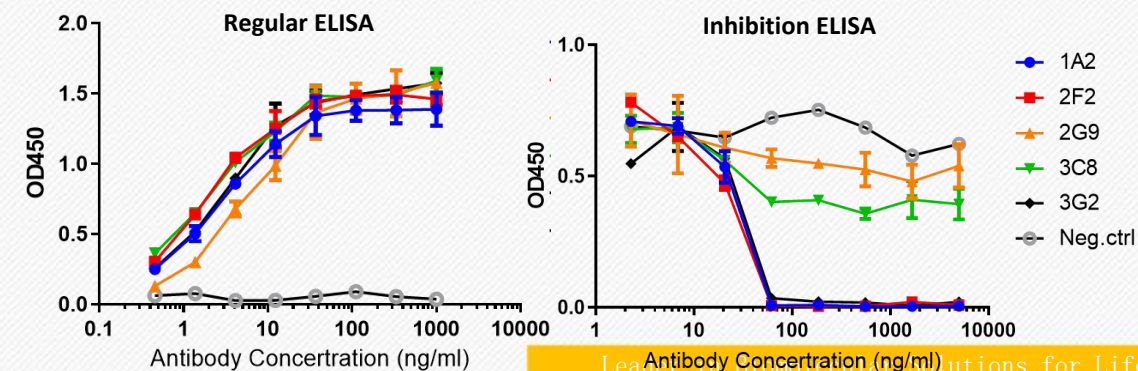
✓ **Type III: non-blocking**

✓ Specific to antibody/antigen complex

✓ Not block antibody-antigen binding

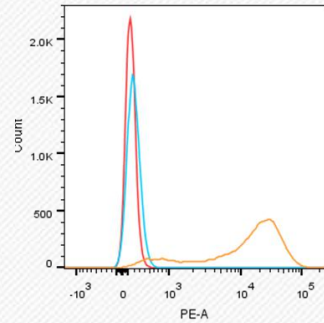
✓ Specifically measure level of antibody/antigen complex in blood

Randomly Pick 5 clones (3 Type 1 & 2 Type 2)

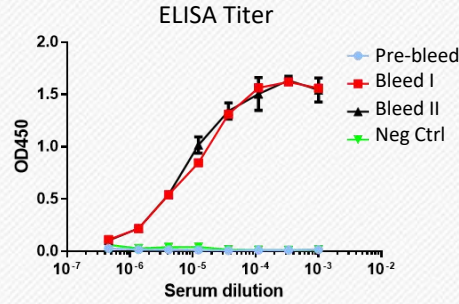


Case 4: Anti-Idiotypic (Anti-ID) Rabbit mAb Development

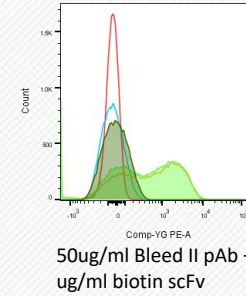
Anti-ID Rabbit Monoclonal Antibodies Development For scFv



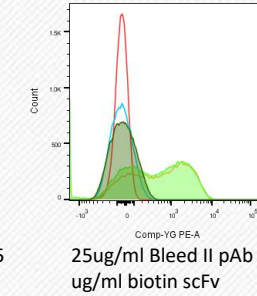
▭ Biotin scFv + PE-streptavidin
▭ PE-streptavidin only
▭ Non-Staining



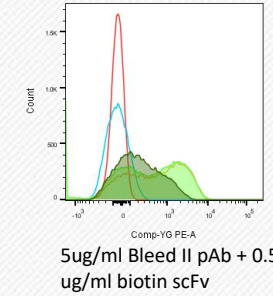
Blocking Activity Measured by FACS



50ug/ml Bleed II pAb + 0.5 ug/ml biotin scFv



25ug/ml Bleed II pAb + 0.5 ug/ml biotin scFv



5ug/ml Bleed II pAb + 0.5 ug/ml biotin scFv

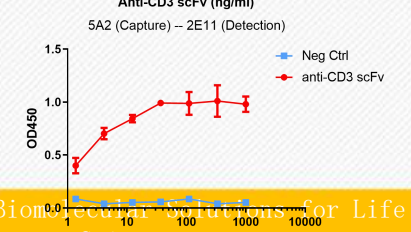
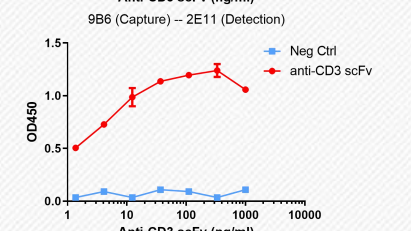
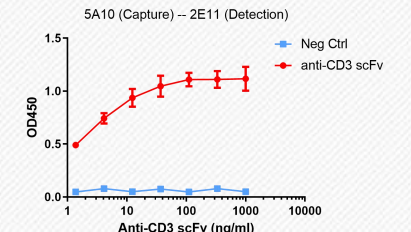
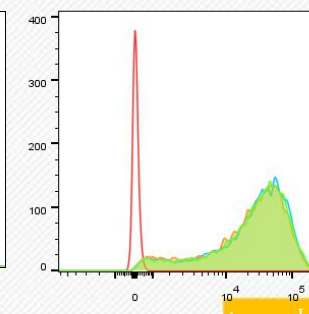
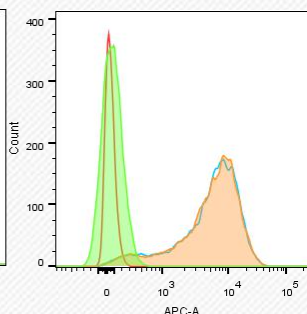
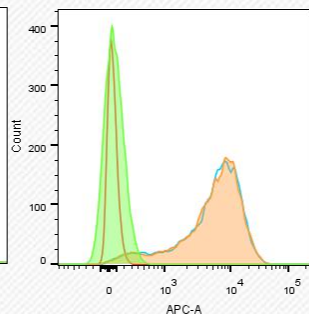
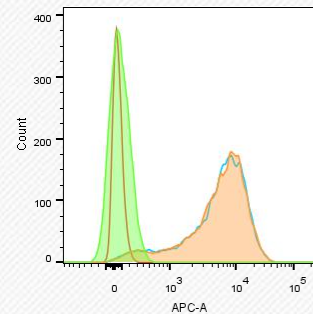
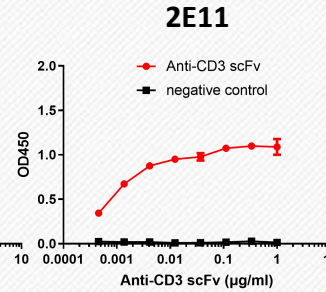
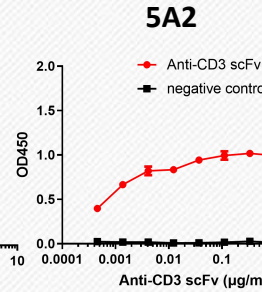
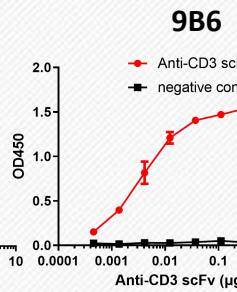
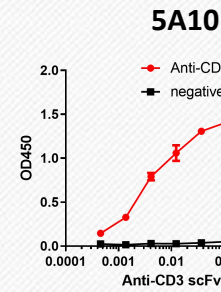
1920 Single B cell clones

192 Positive Clones (rate 10%)

16 blocking anti-ID clones (Type 1)

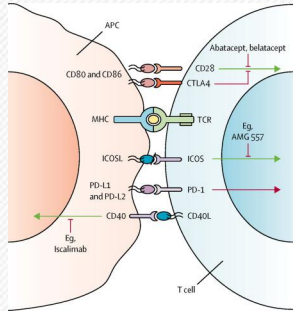
176 non-blocking anti-ID clones (Type 2)

Randomly Pick 4 clones (3 Type 1 & 1 Type 2)

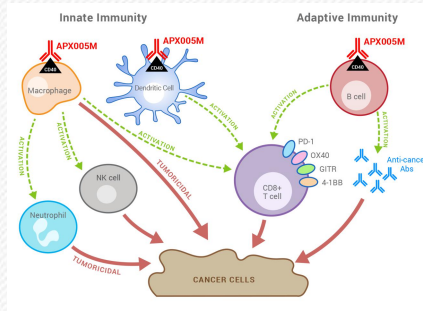


Case 5: Therapeutic Antibody Development

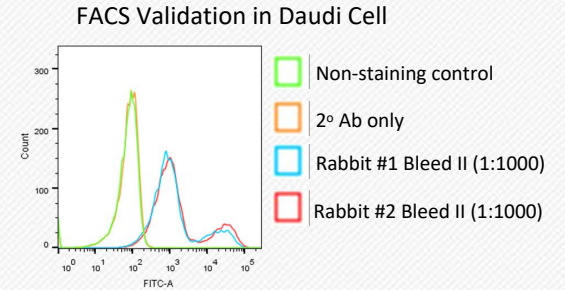
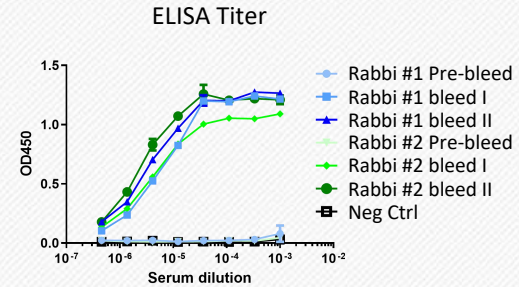
Rabbit Anti-hCD40 Agonist Antibody Discovery



CD40-CD40L Signal Pathway

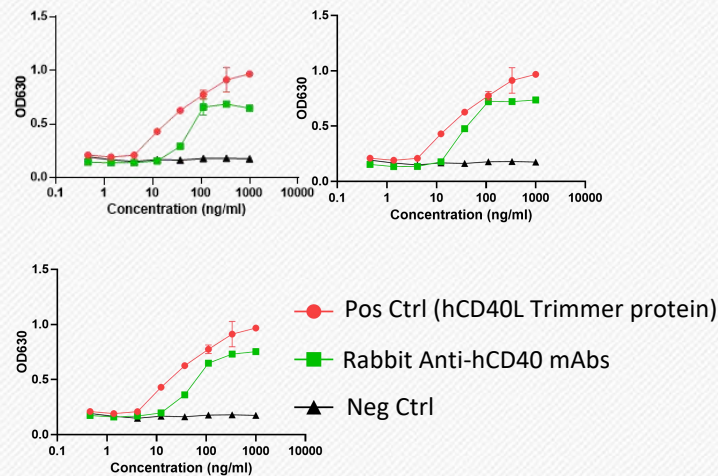


APX005M, first rabbit monoclonal antibody original anti-CD40 therapeutical antibody in Phase II study
<https://www.apexigen.com/>

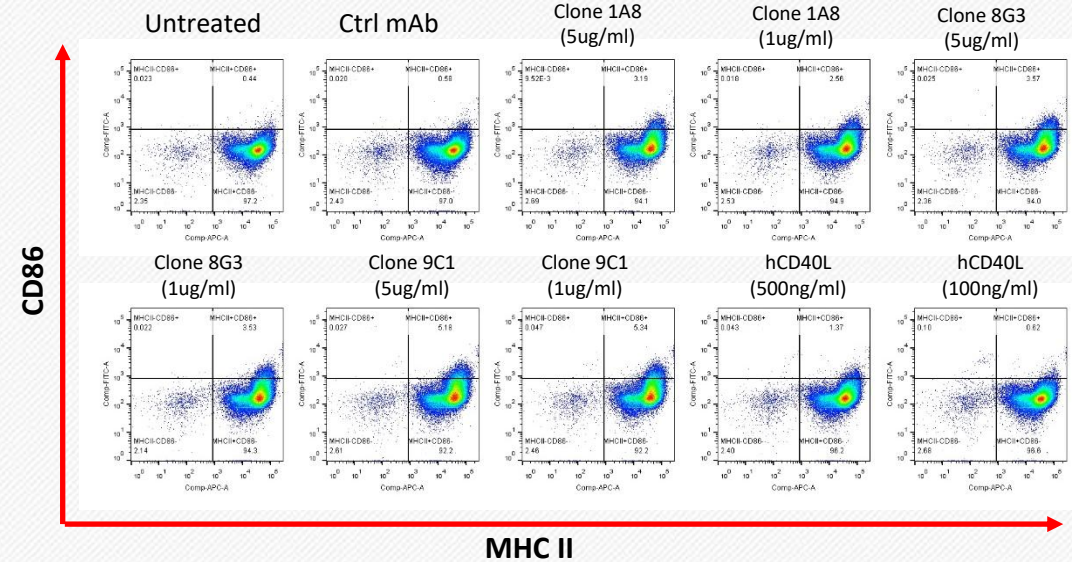


- 1152 Single B cell clones
- 431 positive clones (37%)
- Select 147 B cell sups for further Assay
- 56 FACS clones
- 26 Function clones (Antagonist)

In Vitro Reporter Assay



PMBC Stimulation Assay



A grayscale photograph of a laboratory setting. In the foreground, there are several glass vials and test tubes arranged in a metal rack. A hand in a white glove is visible, holding a pipette and dispensing liquid into one of the vials. The background shows more laboratory equipment and a person in a white lab coat.

Multiplex Assay Platform and Application



Contents

01

Working Principle of Multiplex Assay Platform

02

Merits of Multiplex Assay Platform

03

Applications of Multiplex Assay Platform

Development of Immunoassays

Direction: high-throughput and automatic

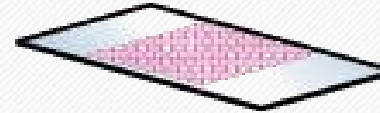
Single tube



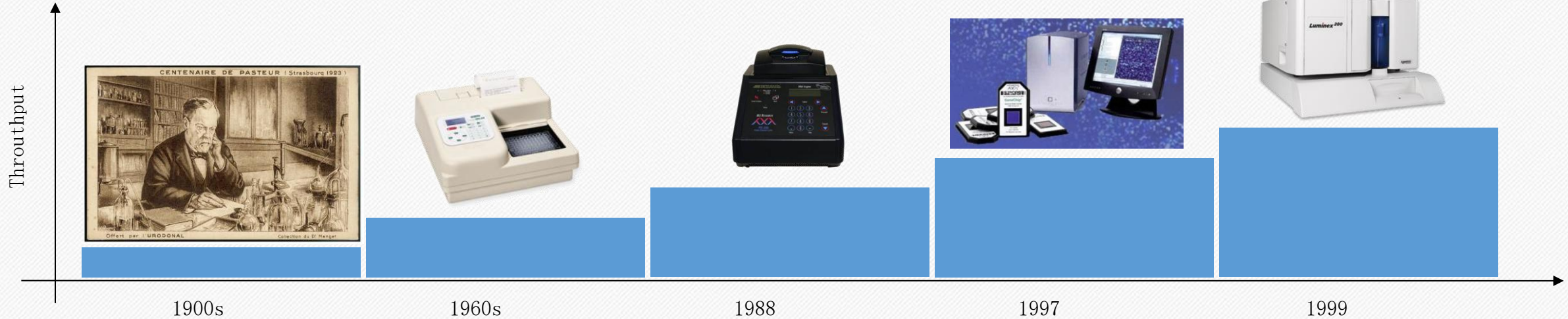
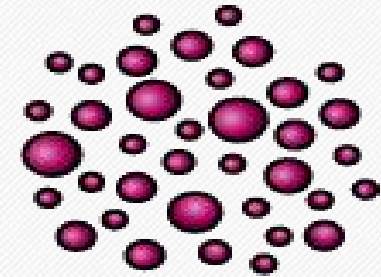
microplate



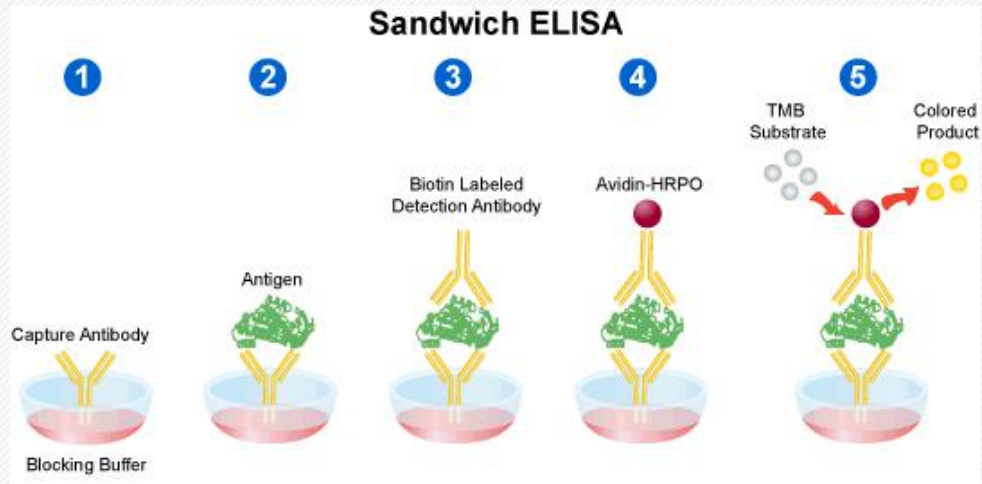
Slide based-chips



Microsphere-based Assay



Working Principle



Traditional sandwich ELISA examines one single target in one well in one test reaction individually

Multiplex assay = multiple ELISA in one well with one same sample

Merits of Multiplex Assay Platform



- 1 **High throughput:** simultaneous quantification of multiple (up to 50) targets in the same sample
- 2 **Less sample usage:** 20-50 μ L fluid sample (culture supernatant, serum, plasma, or other body fluid)
- 3 **Multi-function:** plenty of well-designed and validated multiplex panels meet the needs of broad ranges of research fields
- 4 **High sensitivity:** well-selected antibody pairs with low detection limits (pg/mL level)
- 5 **Broad dynamic range:** compatible with broad expression level of different targets in one sample
- 6 **Good reproducibility:** inter-assay CV <10%
- 7 **Flexibility:** pre-built or custom assay panels



Field of Application



Protein analysis: cytokines, chemokines, growth factors, hormones, secreted proteins, vesicles, etc.



Protein function study: protein co-expression, protein modification, protein interaction.



Biomarker analysis of cancer / cardiovascular diseases / neural disorders



Drug targets screening or validation, evaluation of drug effect

Pre-defined Panels of Multiplex Assay

Pre-defined Panels (Partial)

Panels		Relative indicators
Human	Cytokines (8-plex)	GM-CSF,IFN- γ ,IL-2,IL-4,IL-6,IL-8,IL-10,TNF- α
	Cytokines (17-plex)	G-CSF,GM-CSF,IFN- γ ,IL-1 β ,IL-2,IL-4,IL-5,IL-6,IL-7,IL-8,IL-10,IL-12(p70),IL-13,IL-17A,MCP-1 (MCAF),MIP-1 β ,TNF- α
	Cytokines (27-plex)	FGF basic,Eotaxin,G-CSF,GM-CSF,IFN- γ ,IL-1 β ,IL-1ra,IL-2,IL-4,IL-5,IL-6,IL-7,IL-8,IL-9,IL-10,IL-12 (p70),IL-13,IL-15,IL-17A,IP-10,MCP-1 (MCAF),MIP-1a,MIP-1 β ,PDGF-BB,RANTES,TNF- α ,VEGF
	Chemokines (8-plex)	CCL2/JE/MCP-1,CCL3/MIP-1 alpha,CCL4/MIP-1 beta,CCL5/RANTES,CCL11/Eotaxin,CXCL1/GRO alpha/KC/CINC-1,CXCL10/IP-10/CRG-2,IL-8/CXCL8
	Chemokines (40-plex)	6Ckine/CCL21,BCA-1/CXCL13,CTACK/CCL27,ENA-78/CXCL5,Eotaxin/CCL11,Eotaxin-2/CCL24,Eotaxin-3/CCL26,Fractalkine/CX3CL1,GCP-2/CXCL6,GM-CSF,Gro-a/CXCL1,Gro- β /CXCL2, I-309/CCL1, IFN- γ ,IL-1 β ,IL-2,IL-4,IL-6,IL-8/CXCL8, IL-10,IL-16,IP-10/CXCL10,I-TAC/CXCL11,MCP-1/CCL2,MCP-2/CCL8,MCP-3/CCL7,MCP-4/CCL13,MDC/CCL22,MIF,MIG/CXCL9,MIP-1a/CCL3,MIP-1 δ /CCL15,MIP-3 α /CCL20,MIP-3 β /CCL19,MPIF-1/CCL23,SCYB16/CXCL16,SDF-1 α + β /CXCL12,TARC/CCL17,TECK/CCL25,TNF- α
	Immunotherapy (24-plex)	CCL2/JE/MCP-1,CCL3/MIP-1 alpha,CCL4/MIP-1 beta,CD40 Ligand/TNFSF5,CXCL10/IP-10/CRG-2,GM-CSF,Granzyme B,IFN-alpha,IFN-gamma,IL-1 alpha/IL-1F1,IL-1 beta/IL-1F2,IL-1ra/IL-1F3,IL-2,IL-4,IL-6,IL-8/CXCL8,IL-10,IL-12 p70,IL-13,IL-15,IL-17/IL-17A,IL-33,PD-L1/B7-H1,TNF- α
	T-cell analysis (Th1/Th2, 9-plex)	GM-CSF,IFN- γ ,IL-2,IL-4,IL-5,IL-10,IL-12(p70),IL-13,TNF- α
	T-cell analysis (Th17, 15-plex)	IL-1 β ,IL-4,IL-6,IL-10,IL-17A,IL-17F,IL-21,IL-22,IL-23,IL-25,IL-31,IL-33,IFN- γ ,sCD40L, TNF- α
	T-cell analysis (Treg, 12-plex)	IL-2,IL-19,IL-27 (p28),IL-10,IL-20,IL-28A / IFN- λ 2,IL-12 (p40),IL-22,IL-29 / IFN- λ 1,IL-12 (p70),IL-26,IL-35

Panels		Relative indicators
Mouse	Cytokines (8-plex)	GM-CSF,IFN- γ ,IL-1 β ,IL-2,IL-4,IL-5,IL-10,TNF- α
	Cytokines (23-plex)	IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, Eotaxin, G-CSF, GM-CSF, IFN- γ , KC, MCP-1 (MCAF), MIP-1 α , MIP-1 β , RANTES, TNF- α
	Chemokines (31-plex)	BCA-1/CXCL13,IL-4,MIP-1 α /CCL3,CTACK/CCL27,IL-6,MIP-1 β /CCL4,ENA-78/CXCL5,IL-10,MIP-3 α /CCL20,Eotaxin/CCL11,IL-16,RANTES/CCL5,Eotaxin-2/CCL24,IP-10/CXCL10,MIP-3 β /CCL19,Fractalkine/CX3CL1,I-TAC/CXCL11,SCYB16/CXCL16,GM-CSF,KC/CXCL1,SDF-1 α /CXCL12,I-309/CCL1,MCP-1/CCL2,TARC/CCL17,IFN- γ ,MCP-3/CCL7,TNF- α ,IL-1 β ,MCP-5/CCL12,IL-2,MDC/CCL22
	T-cell analysis (Th1/Th2, 8-plex)	GM-CSF,IFN- γ ,IL-2,IL-4,IL-5,IL-10,IL-12 (p70),TNF- α
	T-cell analysis (Th17, 6-plex)	IFN- γ ,IL-1 β ,IL-6,IL-10,IL-17A,TNF- α
	T-cell analysis (Th17, 10-plex)	sCD40L,IL-17F,IL-21,IL-22,IL-23,IL25-17E,IL-27,IL-31,IL-33,MIP-3 α

* More panels can be selected in our service.

Case Study: Multiplex Assessment of Cytokine Storm

Cytokine 142 (2021) 155478



Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine



Review article

Clinical significance of measuring serum cytokine levels as inflammatory biomarkers in adult and pediatric COVID-19 cases: A review

Benjamin M. Liu ^{a,*}, Thomas B. Martins ^{a,b}, Lisa K. Peterson ^{a,b}, Harry R. Hill ^{a,b,c,d,**}

^a Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, USA

^b ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, USA

^c Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA

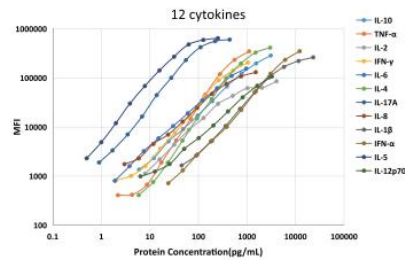
^d Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT, USA



Available panel from ABclonal

Sample Data Report

Standard Curves

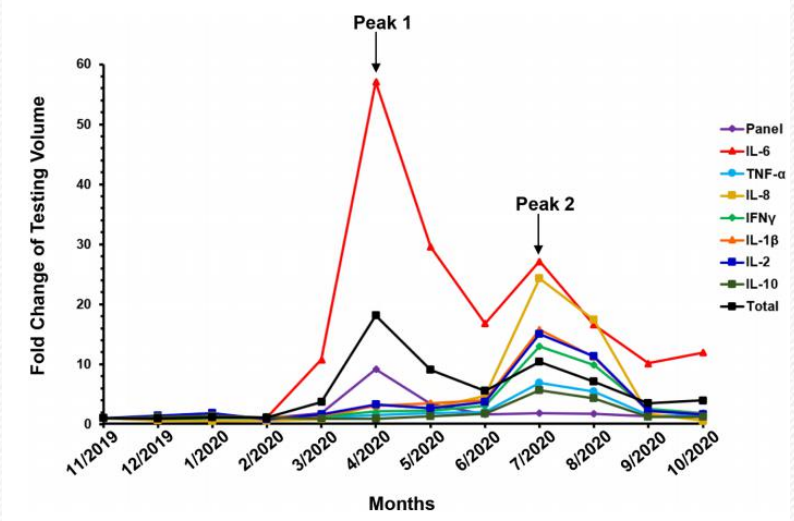
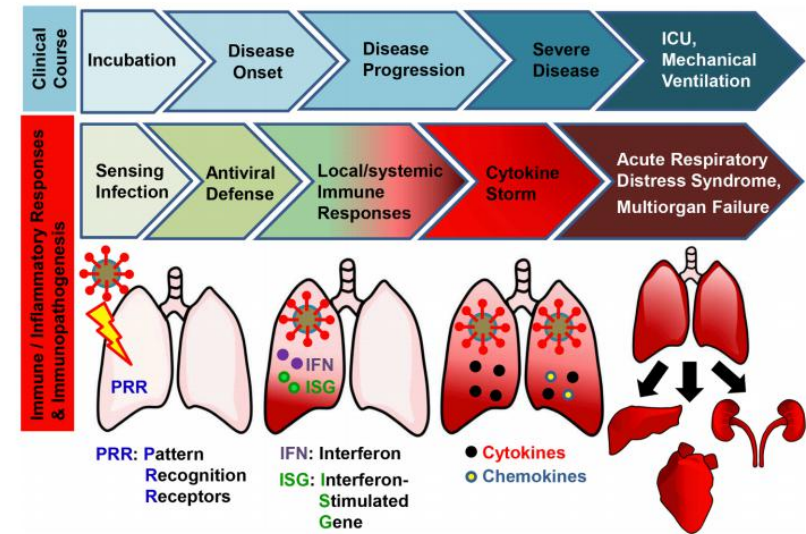


Standard Curves of human cytokine 12-plex panel

Raw Data

	IL-10	MFI	TNF-α	MFI	IL-2	MFI	IFN-γ	MFI	IL-6	MFI	IL-4	MFI
6	1360	2.2	410	8	1130	2	823	1.9	808	5.9	411	
12.1	2340	4.3	418	15.9	2194	4.1	1016	3.8	1594	11.7	759	
24.2	5124	8.7	670	31.8	4375	8.1	1613	7.5	3230	23.5	1885	
48.4	9076	17.3	1898	63.6	8788	16.3	3619	15.1	5904	46.9	5287	
96.8	17137	34.7	5342	127.3	15258	32.6	7617	30.2	10452	93.9	15003	
193.5	33715	69.3	14722	254.5	29793	65.1	16821	60.3	19076	187.8	37248	
387	67752	138.6	45534	509	43202	130.3	41673	120.6	35170	375.5	99143	
774	122046	277.3	119833	1018	61981	260.5	89515	241.3	61545	751	197126	
1548	198223	554.5	234012	2036	63198	521	142440	482.5	110648	1502	328525	
3096	286796	1109	347112	4072	85285	1042	206014	965	155021	3004	413160	

* MFI: mean fluorescence intensity



Case Study: Multiplex Assessment of SARS-CoV-2 Abs

Clinical Biochemistry 97 (2021) 54–61



Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem



Multiplex assessment of SARS-CoV-2 antibodies improves assay sensitivity and correlation with neutralizing antibodies

Nathan Cook^a, Lingqing Xu^b, Shaymaa Hegazy^a, Bradley J. Wheeler^c, Adam R. Anderson^d, Nancy Critelli^d, Mary Yost^a, Anita K. McElroy^b, Michael R. Shurin^{a,e}, Sarah E. Wheeler^{a,f,*}

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^c University of Pittsburgh, School of Computing and Information, Pittsburgh, PA, USA

^d Bio-Rad Laboratories, Inc., Hercules, CA, USA

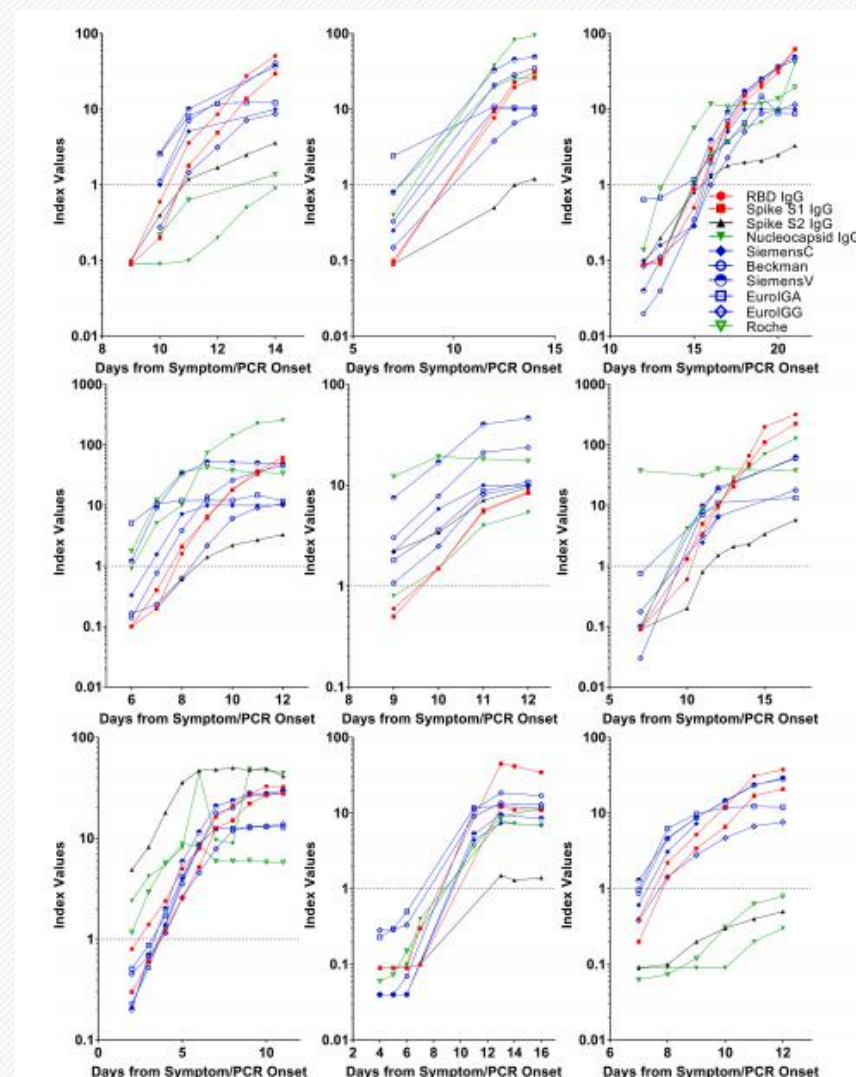
^e University of Pittsburgh, Departments of Pathology and Immunology, Pittsburgh, PA, USA

^f University of Pittsburgh, Department of Pathology, Pittsburgh, PA, USA

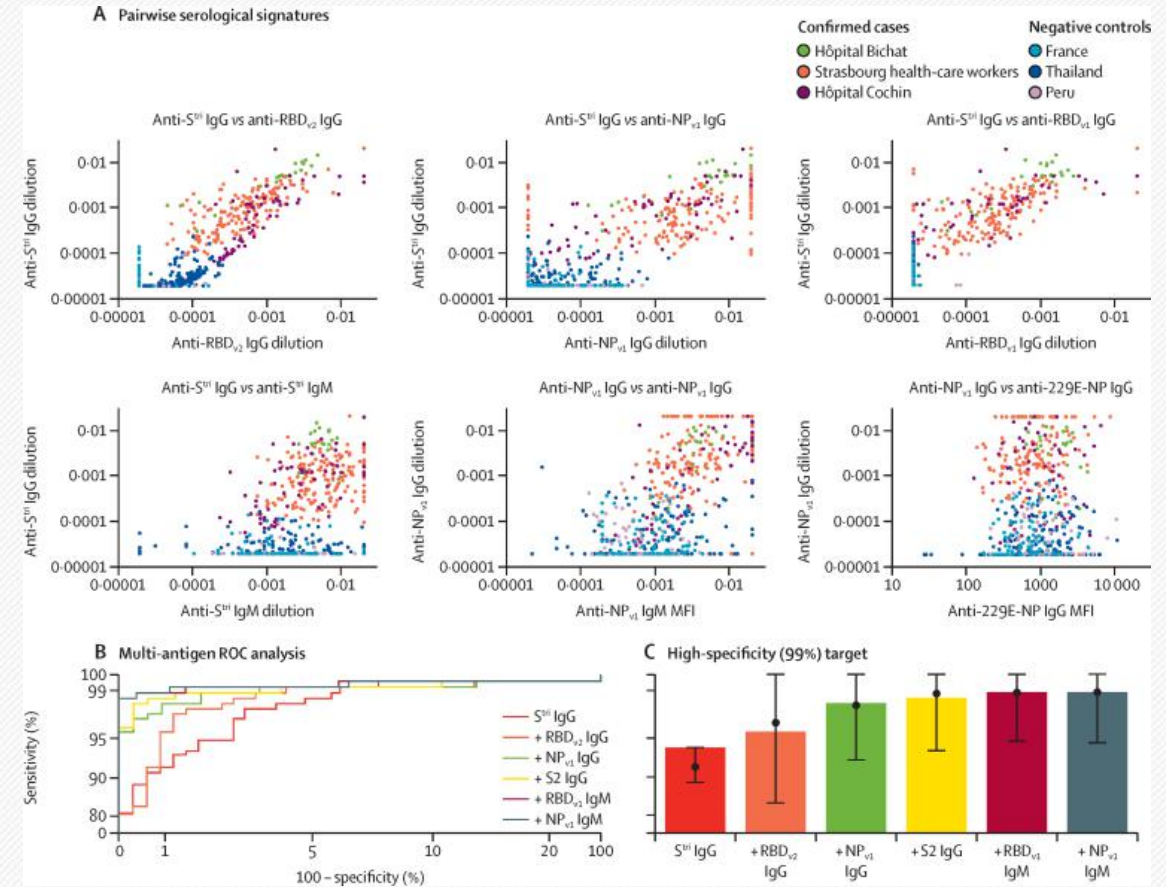
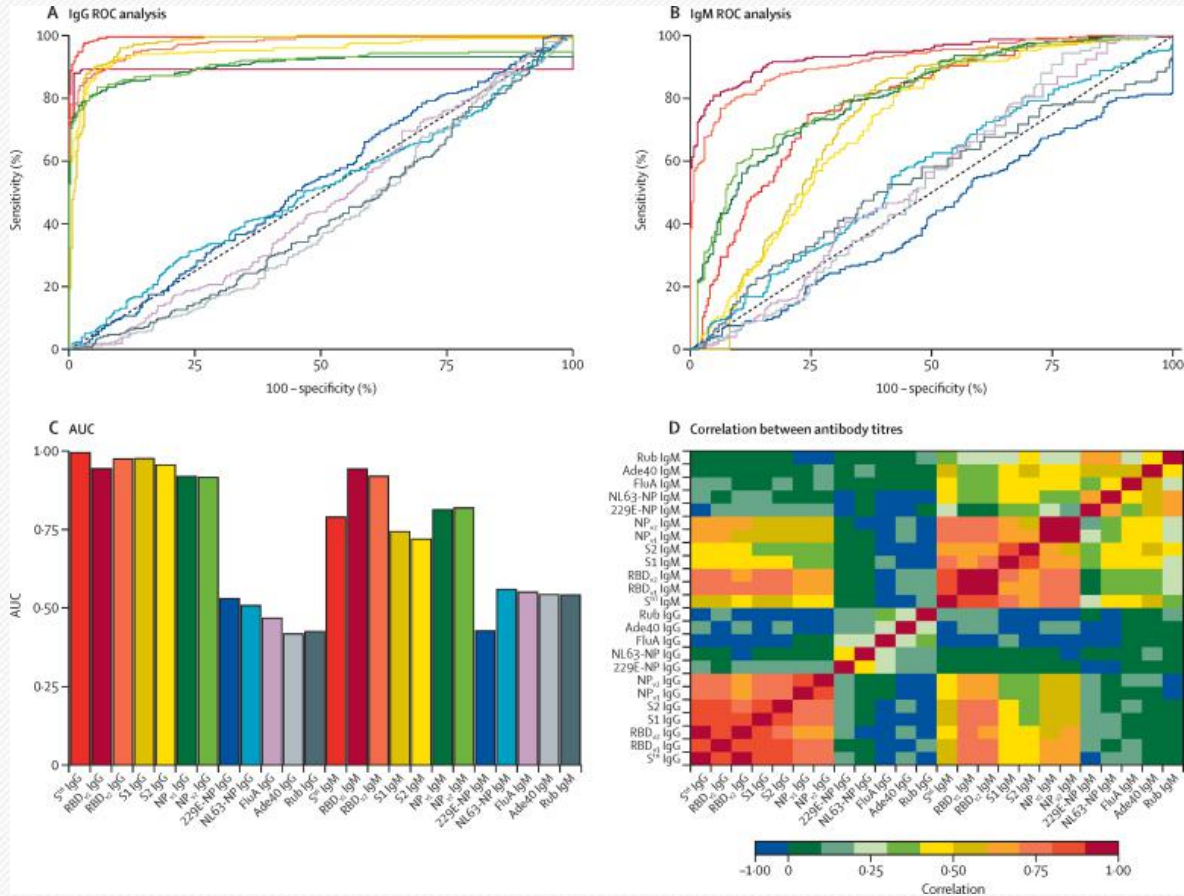
Table 1
SARS-CoV-2 antibody assay details.

Assay	Abbreviation	Units	Positive cutoff	Antibody	Antigen	Analyzer	Manufacturer	Manufacturer information
SARS-CoV-2 IgG Panel	BR	U/mL	≥10	IgG	RBD, S1, S2, N	BioPlex 2200	Bio-Rad Laboratories, Inc	Hercules, CA, USA
Access SARS-CoV-2 IgG	Beckman	S/CO	≥1.0	IgG	RBD	UniCel DxI 800	Beckman Coulter	Brea, CA, USA
COV2T Centaur	SiemensC	Index	≥1.0	total antibody	RBD	Advia Centaur XP	Siemens Healthineers	Erlangen, Germany
COV2T Vista	SiemensV	QUAL	≥1000	total antibody	RBD	Dimension Vista 1500	Siemens Healthineers	Erlangen, Germany
Anti-SARS-CoV-2 ELISA IgG	EuroIG	Index	≥1.1	IgG	S1 ^a	Manual ELISA	Euroimmun	Lubeck, Germany
Anti-SARS-CoV-2 ELISA IgA	EuroIGA	Index	≥1.1	IgA	S1 ^a	Manual ELISA	Euroimmun	Lubeck, Germany
Elecsys Anti-SARS-CoV-2	Roche	COI	≥1.0	total antibody	N	Cobas e 411	Roche Diagnostics	Basel, Switzerland

^a May include RBD.



Case Study: Multiplex Assessment of SARS-CoV-2 Abs



Lancet Microbe. 2021 Feb;2(2):e60-e69. doi: 10.1016/S2666-5247(20)30197-X.

COVID-19 related ELISA Kits



Target	Cat. No.	Product Name	Sensitivity	Assay Range
Spike S1-RBD	RK04135	SARS-COV-2 Spike RBD Protein ELISA Kit	6.32 pg/mL	15.6-1000pg/ml
Spike S1-RBD	RK04137	SARS-COV-2 Spike RBD Protein IgG Antibody ELISA Kit	Qualitative analysis	Qualitative analysis
Spike S1-RBD	RK04144	SARS-COV-2 Spike RBD Protein Antibody ELISA Kit	Qualitative analysis	Qualitative analysis
Spike S1-RBD	RK04149	SARS-CoV-2 Inhibitor Screening Kit (RBD)	0.112 ug/mL	0.312-20ug/ml
Spike S1	RK04138	SARS-COV-2 Spike S1 Protein IgG Antibody ELISA Kit	Qualitative analysis	Qualitative analysis
Spike S1	RK04145	SARS-COV-2 Spike S1 Protein Antibody ELISA Kit	157.2 pg/mL	0.78-50ng/ml
Spike S1	RK04154	SARS-CoV-2 Spike S1 Protein ELISA Kit	32.22 pg/mL	78.1-5000pg/ml
S1+S2 ECD (S-ECD)	RK04156	SARS-COV-2 S1+S2 ECD (S-ECD) Protein IgG ELISA Kit	Qualitative analysis	Qualitative analysis
S1+S2 ECD (S-ECD)	RK04158	SARS-CoV-2 S1+S2 ECD (S-ECD) Protein Antibody ELISA Kit	1.22 ng/mL	3.12-200 ng/mL
S1+S2 ECD (S-ECD)	RK04159	SARS-CoV-2 S1+S2 ECD (S-ECD) Protein ELISA Kit	11.65 pg/mL	39.1-2000pg/ml
Nucleocapsid Protein	RK04136	SARS-COV-2 Nucleocapsid Protein ELISA Kit	3.11 pg/mL	7.8-500pg/ml
Nucleocapsid Protein	RK04139	SARS-COV-2 Nucleocapsid Protein IgG Antibody ELISA Kit	Qualitative analysis	Qualitative analysis

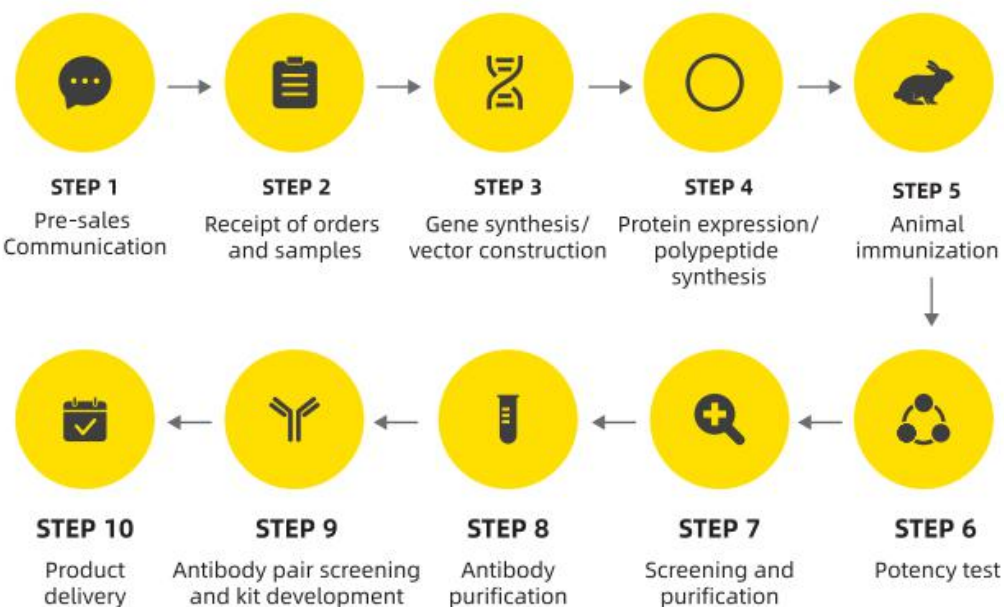
Antibody Pairs for Immunoassays

Target	Cat. No.	Product Name	Application	Reactivity
Spike S1-RBD	RM01763	SARS-COV-2 Spike RBD Protein Antibody (Capture Ab)	ELISA	SARS-CoV-2
Spike S1-RBD	RM01764	SARS-COV-2 Spike RBD Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1	RM17484	SARS-COV-2 Spike S1 Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1	RM17501	SARS-COV-2 Spike S1 Protein Antibody (Capture Ab)	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17505	SARS-CoV-2 Nucleocapsid Protein Antibody (Capture Ab)	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17507	Biotinylated SARS-CoV-2 Nucleocapsid Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1-RBD	RM17561	Biotinylated SARS-COV-2 Spike RBD Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1	RM17568	SARS-COV-2 Spike S1 Protein Antibody (Capture Ab)	ELISA	SARS-CoV-2
Spike S1	RM17569	SARS-COV-2 Spike S1 Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1	RM17570	Biotinylated SARS-COV-2 Spike S1 Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17574	SARS-COV-2 Nucleocapsid Protein Antibody (Capture Ab)	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17575	SARS-COV-2 Nucleocapsid Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17576	Biotinylated SARS-CoV-2 Nucleocapsid Protein Antibody (Detection Ab)	ELISA	SARS-CoV-2
Spike S1-RBD	RM17580	Anti-SARS-CoV-2 Spike RBD Control Antibody, Chimeric mAb	ELISA	SARS-CoV-2
Spike S1	RM17582	Anti-SARS-CoV-2 Spike S1 Control Antibody, Chimeric mAb	ELISA	SARS-CoV-2
Nucleocapsid Protein	RM17584	Anti-SARS-CoV-2 Nucleocapsid Control Antibody, Human mAb	ELISA	SARS-CoV-2

Antibody Pairs for Immunoassays

Target	Cat. No.	Product Name	Application	Reactivity
IL-2	RM17589	Human IL-2 Monoclonal Antibody, Rabbit mAb (CAP)	ELISA	Human
IL-2	RM17590	Human IL-2 Monoclonal Antibody, Rabbit mAb (DET)	ELISA	Human
IL-6	RM17601	Human IL-6 Monoclonal Antibody, Rabbit mAb (CAP)	ELISA	Human
IL-6	RM17602	Human IL-6 Monoclonal Antibody, Rabbit mAb (DET)	ELISA	Human
IL-6	RM17603	Biotinylated Human IL-6 Monoclonal Antibody, Rabbit mAb (DET)	ELISA	Human
IL-8	RM17621	Human IL-8 Antibody, Goat Ab (CAP)	ELISA	Human
IL-8	RM17622	Human IL-8 Antibody, Goat Ab (DET)	ELISA	Human
IL-8	RM17625	Biotinylated Human IL-8 Antibody, Goat Ab (DET)	ELISA	Human
AMH	RM17623	Human AMH/MIS Monoclonal Antibody, Rabbit mAb (CAP)	ELISA	Human
AMH	RM17624	Human AMH/MIS Monoclonal Antibody, Rabbit mAb (DET)	ELISA	Human

Custom Services for Immunoassay Development



Service Cases

>> Case 1 Combined Development of GPCR Antibody Pair and ELISA KIT

G Protein-Coupled Receptors (GPCRs) are a large class of membrane protein receptors. GPCRs are the largest family of membrane receptor proteins in humans and play an important role in cell signal transduction. GPCRs are closely related to human diseases, and more than 40% of drugs currently target GPCRs. Because GPCRs contain multiple transmembrane domains, it is difficult to obtain stable and intact GPCR proteins and its research is extremely challenging and important. In this project, multiple extracellular regions are used as immunogens, and rabbit polyclonal antibodies and murine monoclonal antibodies are tested in parallel to screen the matched antibody pairs, laying a solid foundation for clinical diagnosis. MRGPRX2 is a key receptor for drug hypersensitivity reaction and is a member of the GPCR family. The developed ELISA Kit has high sensitivity and specificity and is suitable for the testing of blood samples.

Diagnostic value of target:

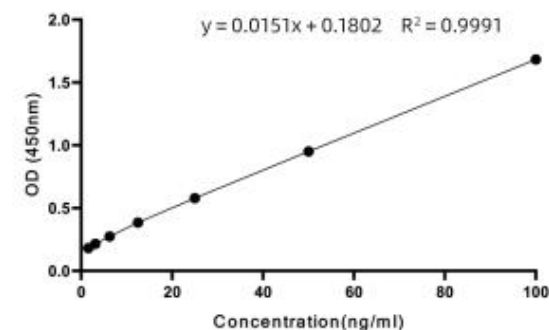
The key receptor for drug hypersensitivity reaction.

Application: MRGPRX2 test, for companion diagnosis

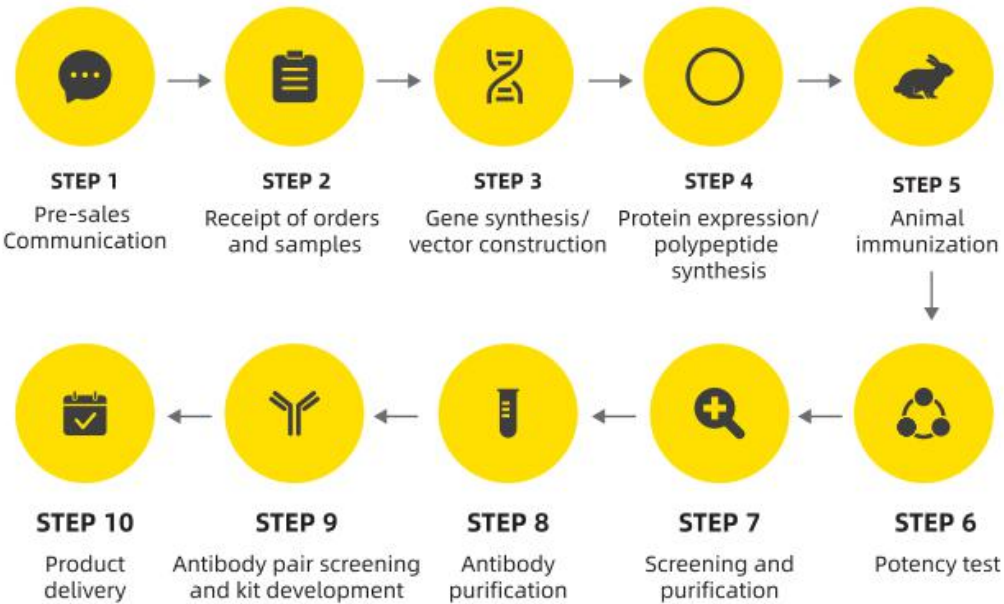
Antigens: multiple recombinant proteins and peptides

Validation: recombinant proteins and peptides

Screening: rabbit polyclonal antibody-mouse monoclonal antibody pairs



Custom Services for Immunoassay Development



>> Case 2 Combined Development of AFP Antibody Pair and ELISA KIT

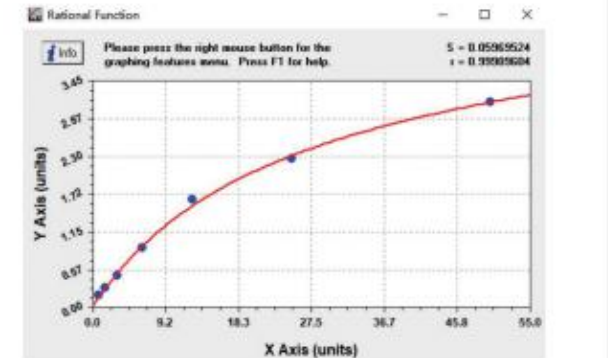
Alpha-fetoprotein (AFP) is one of the most commonly used tumor markers in clinic. About 60% of patients with primary hepatocarcinoma were found with elevated AFP level in serum. AFP is closely related to the tumorigenesis and development of liver cancer and many other tumors. It can be used as a predictor for many tumors. At present, it is mainly used as a serum marker for the diagnosis of primary liver cancer and the monitoring of treatment efficacy. The developed AFP murine monoclonal antibodies have high specificity and sensitivity and good stability, with detection limit as low as 0.215 ng/mL.

Application: AFP diagnostic antibody pair development

Antigens: recombinant proteins

Validation: recombinant Proteins

Screening: mouse monoclonal antibody-mouse monoclonal antibody pairs



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