

Antibody Drug Development: Challenges & Solutions

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Antibody Drug Market



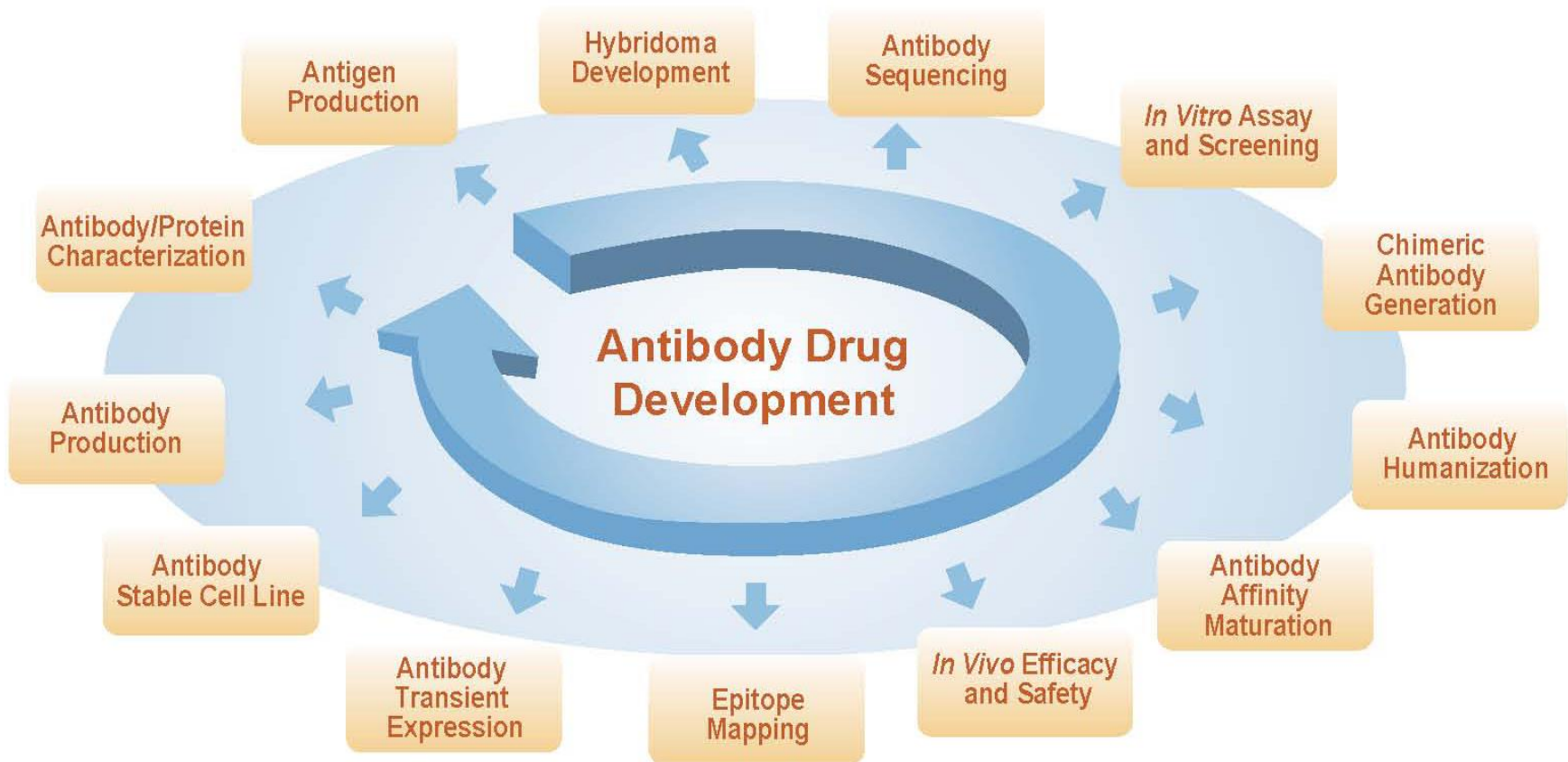
Table 3 The 20 top-selling biopharmaceutical products in 2013

Ranking	Product	Sales (\$ billions) ^a	Year first approved	Company	Patent expiry (EU)	Patent expiry (US)
1	Humira (adalimumab; anti-TNF)	11.00	2002	AbbVie & Eisai	2018	2016
2	Enbrel (etanercept; anti-TNF)	8.76	1998	Amgen, Pfizer, Takeda Pharmaceuticals	2015	2028
3	Remicade (infliximab; anti-TNF)	8.37	1998	J&J, Merck & Mitsubishi Tanabe Pharma	2015	2018
4	Lantus (insulin glargine)	7.95	2000	Sanofi	2014	2014
5	Rituxan/MabThera (rituximab; anti CD20)	7.91	1997	Biogen-IDEC, Roche	2013	2016
6	Avastin (bevacizumab; anti-VEGF)	6.97	2004	Roche/Genentech	2019	2017
7	Herceptin (anti-HER2)	6.91	1998	Roche/Genentech	2014	2019
8	Neulasta (pegfilgrastim)	4.39	2002	Amgen	2015	2014
9	Lucentis (ranibizumab; anti-VEGF)	4.27	2006	Roche/Genentech, Novartis	2016	2016
10	Epogen/Procrit/Eporex/ESPO (epoetin alfa)	3.35	1989	Amgen, J&J, KHK	Expired	2013
11	Novolog/Novorapid (insulin aspart)	3.13	1999	Novo	2015	2015
12	Avonex (IFN- β -1a)	3.00	1996	Biogen Idec	2015	2015
13	Humalog mix 50:50 (insulin lispro)	2.61	1996	Lilly	2015	2014
14	Rebif (IFN- β -1a)	2.59	1998	Merck Serono	2015	2013
15	Aranesp/Nesp (darbepoetin α)	2.42	2001	Amgen, KHK	2016	2024
16	Advate/Recombinate (Octocog α)	2.37	1992	Baxter		
17	Levemir (insulin detemir)	2.15	2004	Novo	[Levemir]	2014
18	Actrapid/Novolin (insulin)	2.02	1991	Novo	2017	
19	Erbix (cetuximab; anti-EGF)	1.92	2004	Bristol-Myers Squibb, Merck Serono	2014	2016
20	Eylea (aflibercept; anti-VEGF)	1.88	2011	Regeneron, Bayer	2020	2021

^aFinancial data from LaMerie Business Intelligence. J&J, Johnson & Johnson

Gary Walsh, 2014, Nature Biotechnology

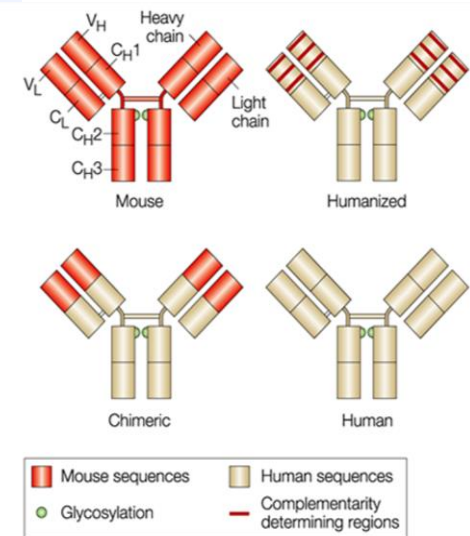
Antibody Drug Development Process





Technologies to Generate Therapeutic Antibodies

- ◆ Hybridoma with human transgenic mice >>>> Human antibody
- ◆ Hybridoma with B-cells from immunized human body >>>> Human antibody
- ◆ **Hybridoma with rodent system >>>> Humanized antibody**
- ◆ SLAM technology >>>> Humanized antibody
- ◆ Phage/yeast display >>>> Human/Humanized antibody
- ◆ Next generation antibody sequence >>>> Human/Humanized antibody



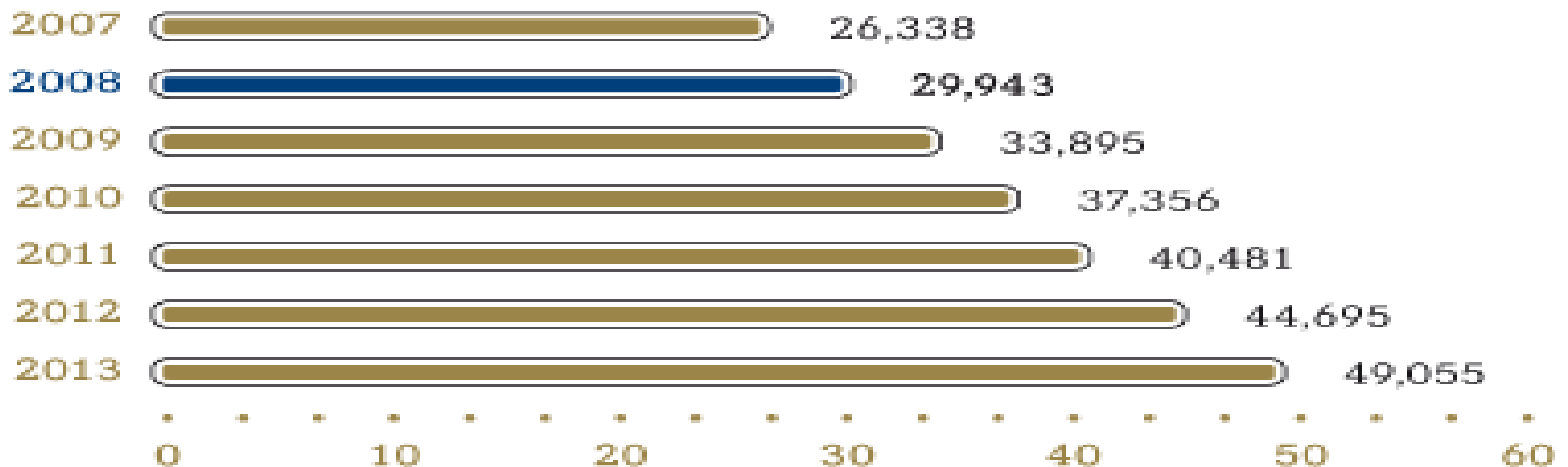
Nature Reviews | Cancer

Hybridoma Technology – Old but Powerful



- By 2015, FDA has approved 44 therapeutic antibody drugs.
- 41 out of 44 were derived from hybridoma technology, 3 from phage-display technology.
- The hybridoma technology generates high market value.

SALES OF MARKETED THERAPEUTIC ANTIBODIES 2007-2013
(Datamonitor, in million US\$)



Hybridoma - The Critical Path for Ab Lead Identification



Reagent
preparation

Animal
Immunization

Assay
Development

Hybridoma library: $1-2 \times 10^4$ clones in 25 plates/ fusion, total 4-8 fusions

1°. *In vitro* target binding assay (selectivity against family members)

2°. Cell based blocking assay

3°. Cell based functional assay

Hit subcloning and characterization

Lead selection

Animal model study
(efficacy)

Target engagement, PK and
safety assessment

Lead optimization
(Humanization, affinity maturation if necessary)

GenScript's Expertise on Hybridoma Development



Conventional Hybridoma Generation Procedures

Immunogen Preparation



Immunization



Fusion



Hybridoma Screening



Sub-cloning



Hybridoma Expansion, Purification and Cell Line Freezing

GenScript's Expertises

Multiple approaches on immunogen preparation

Variety of choices on host animals

Electro-fusion with high fusion efficiency ($> 1/5000$)

High throughput screening for the binders against the most native epitopes

Project management and characterization of a panel of binders

Choices on Immunogens



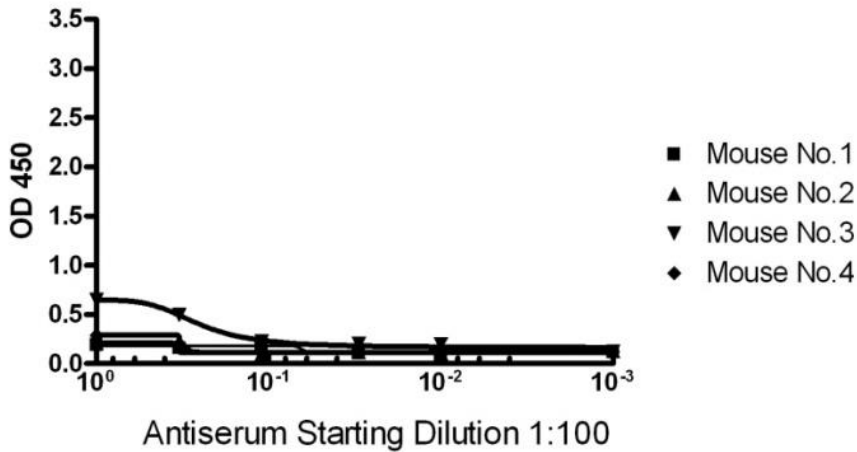
- **Peptide:** Native or modified
- **Protein:** secreted proteins, extracellular domain of membrane proteins, membrane preps
- **Whole cell:** Receptors including GPCRs; surface antigens including immune checkpoint proteins, ion channels and transporters
- **DNA:** Plasmids
- **Virus-like particles**
- **Combination** of the above

ImmunoPlus™ Breaking Immune Tolerance

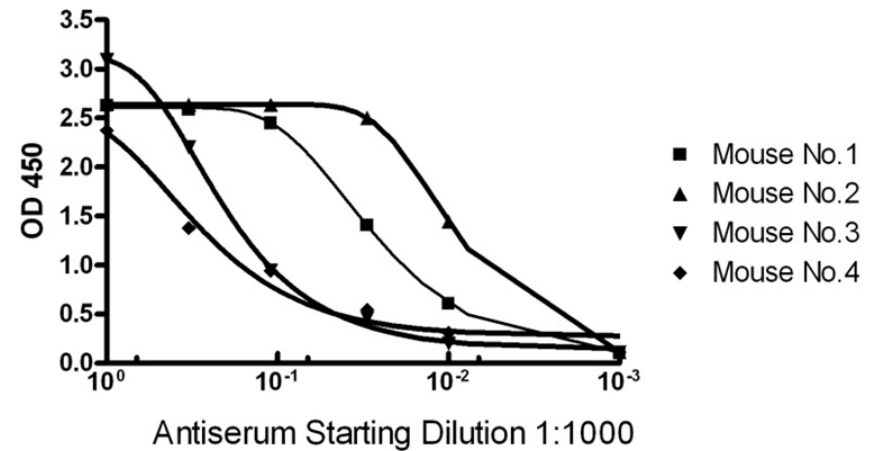


- ImmunoPlus™: A powerful tool to break immune tolerance and enhance Antigenicity
- Ideal for raising antibodies against antigens of high degree of homology between human and rodent counterparts

Regular Immunization Group



Immunization Group with ImmunoPlus Technology

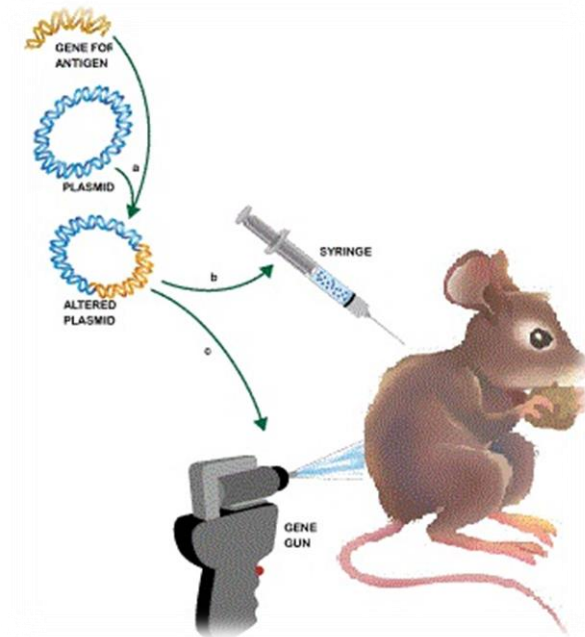


Surrogate antibody development for target validation with 96% homology between human and mouse antigen proteins.

Gene Immunization (DNA Immunization)



- ◆ Gene immunization is a powerful method to generate mAbs against membrane-bound proteins such as GPCRs and ion channel proteins that are hard to express, purify and prepare as immunogens .
- ◆ Host animals are injected with plasmid encoding target proteins.
- ◆ Target protein expressed *in vivo*, eliciting the immune response.



Gene Immunization Options

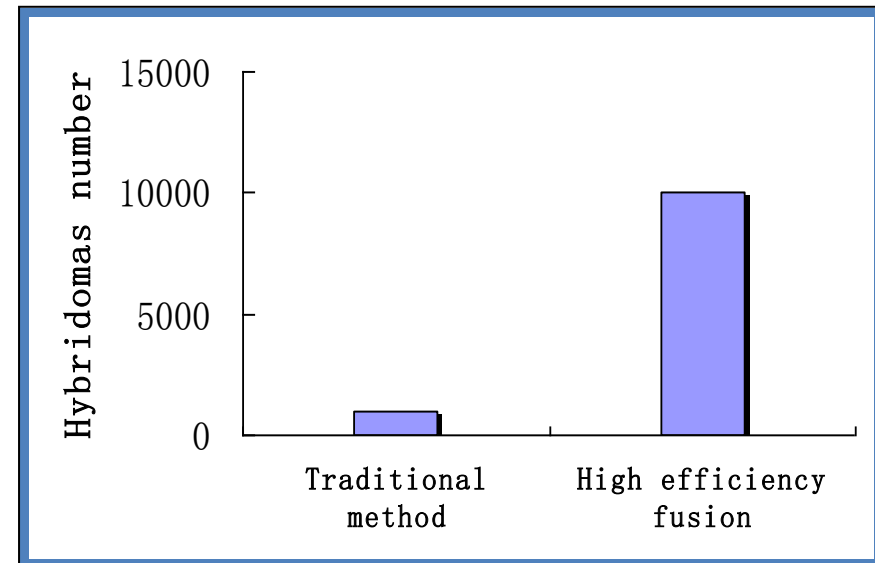


Items	Option
Immunogen	Plasmid, Plasmid + cells expressing target protein, Plasmid + target protein,
Species	Balb/c mouse, C57 black mouse, Wistar rat, GANP mouse;
Route	Gene gun-mediated DNA delivery;
Dose per animal	3-15 μg DNA 5 x 10 ⁶ cell or 10 μg protein boost;

Immunization and Hybridoma Fusion



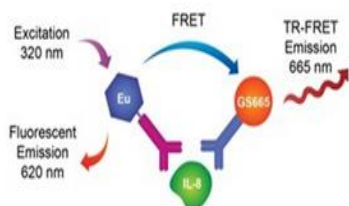
- Multiple choices of animal strains
 - ✓ Mouse: Balb/c, C57Bl/6, MRL, C3H/He, SJL and GANP transgenic mouse
 - ✓ Rat: Wistar and Sprague Dawley
- Reproducible high-titer in most cases
 - ✓ By average 1:512,000
- Electrofusion
 - ✓ High cell-fusion efficiency to increase the size of library



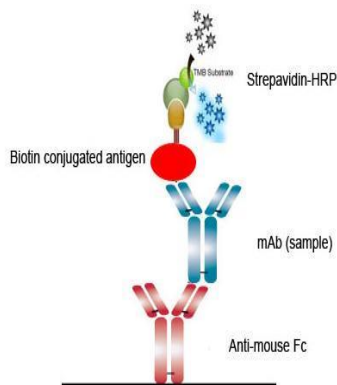
High-Throughput Binder Screening



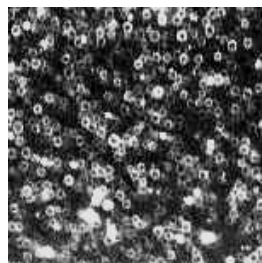
- Natural epitope-based
 - ✓ Soluble target: ELISA (capture and indirect), TR-FRET
 - ✓ Membrane target: FACS and FMAT



Homogeneous (HT March™) binding assay system



NativeSelect™ screening



Cells expressing recombinant target



Parental cells



BD FACSCalibur with HTS loader

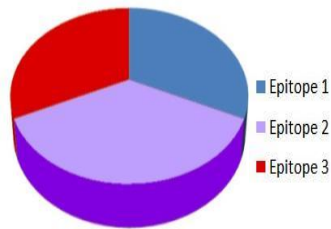


HTS iQue™ Screening System

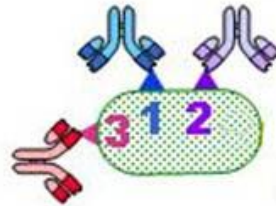
Antibody Characterization



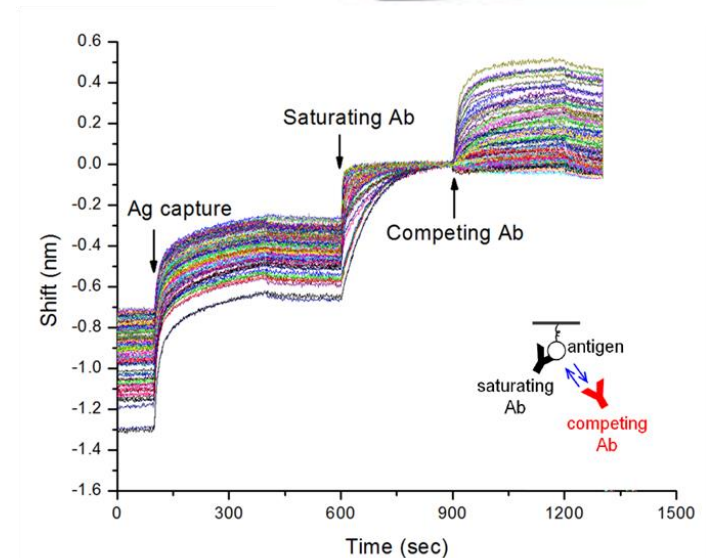
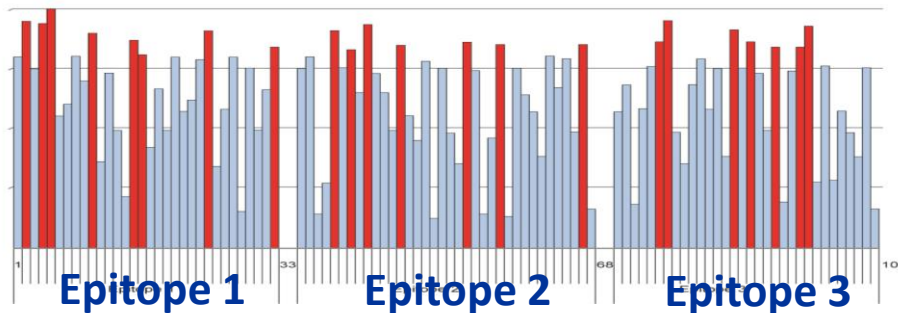
- **Epitope binning** (ELISA or SPR based)
- **Relative affinity ranking** (K_{off} by Biacore or ELISA)
- **Affinity measurement** (Biacore T200)
- **IC50 or EC50** determination in cell-based functional assays
- **Target specificity and species cross-reactivity**



■ Epitope 1
■ Epitope 2
■ Epitope 3



Relative Affinity

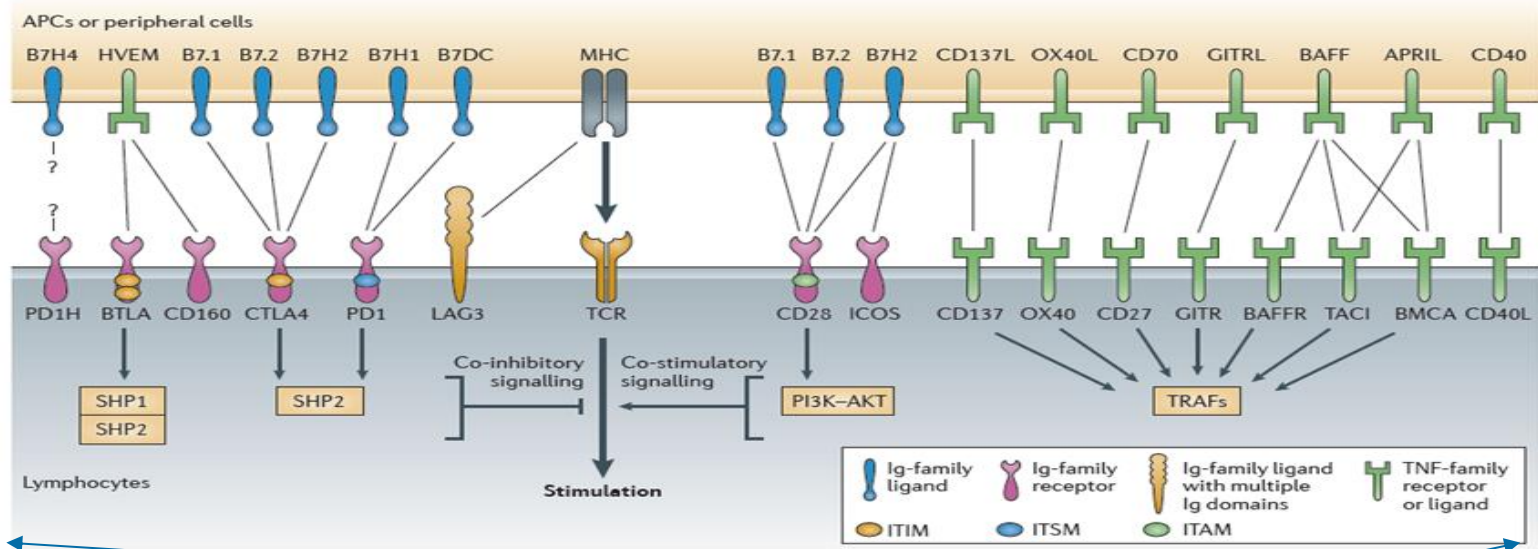


Epitope binning by Octet RED 96

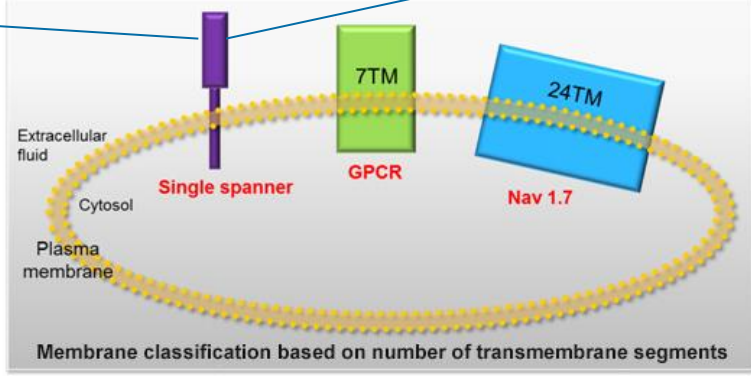


- ◆ Immunization portfolios
 - Multiple immunogen choices;
 - Optimal immunization schedule;
 - ImmunoPlus™ to break immune tolerance
- ◆ High throughput screening platform
 - Binding screening: capture ELISA for soluble target and HT FACS for membrane target
 - Functional screening: 96-well/384-well plate, liquid handling
- ◆ Assay platform (*in vitro* & *in vivo*)-
 - Custom assay development in 1-2 months
- ◆ Humanization & affinity maturation
 - Affinity guaranteed humanization;
 - FASEBA high-content screening;
- ◆ Cell line and process development
 - Guaranteed titer > 2g/L
 - In-house developed expression system

Raising Antibodies to Membrane Proteins



We experienced many successful cases using ECD proteins, membrane preps, recombinant cells, plasmid DNAs alone or their combinations as immunogens to maximize the chance of success

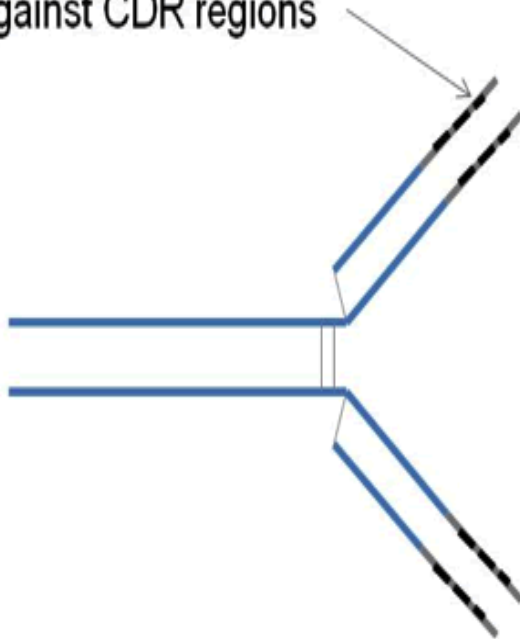


Anti-Idiotypic Antibody

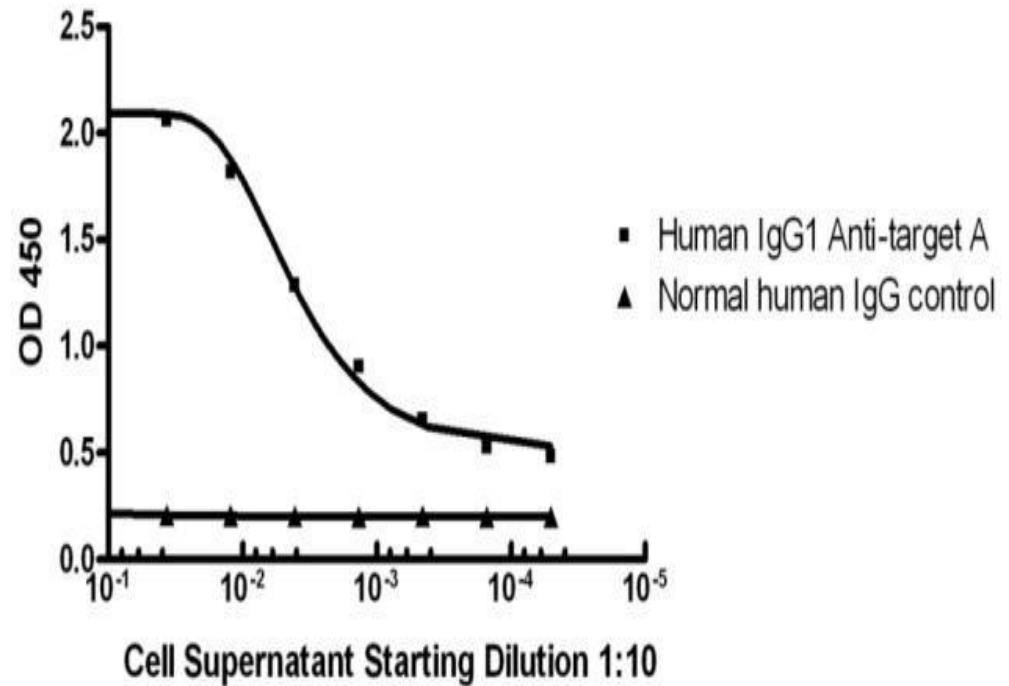


Anti-Idiotypic

Directed against CDR regions



Anti-idiotypic mAb ELISA Test

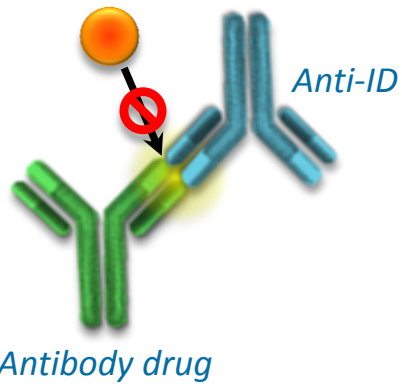


Idiotypic (*antigen specific*)– the antigen binding specificity defined by the distinctive sequence in the variable region of antibodies

Different Types of Anti-IDs

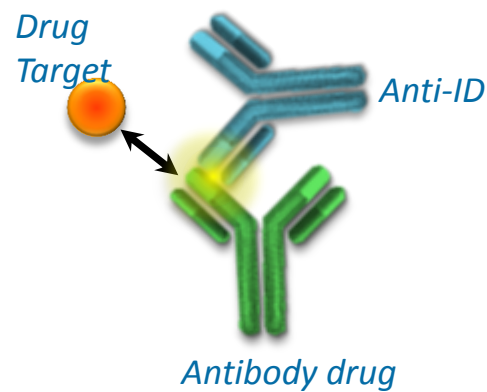


Antigen-blocking



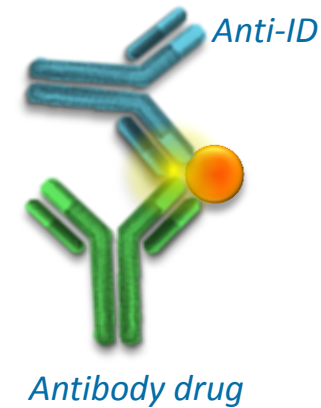
- Paratope-specific
- Inhibitory
- Neutralizing
- Detects free drug

Non-blocking



- Not paratope-specific
- Not inhibitory
- Detects total drug (free, partially bound, fully bound)

Complex-specific



- Drug-target complex specific
- Not inhibitory
- Detects bound drug only



1

Pharmacokinetic (PK) Assays

- Used to measure the antibody drug level in patient samples

2

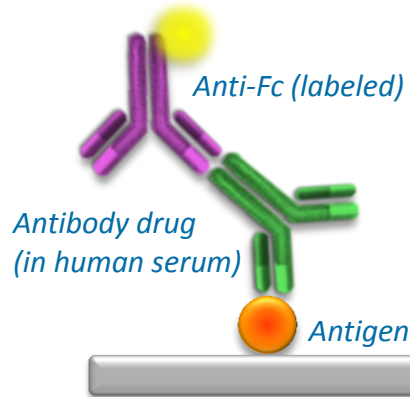
Immunogenicity (Anti-Drug Antibody) Assays

- Used as a positive control or reference standard for ADA measurement

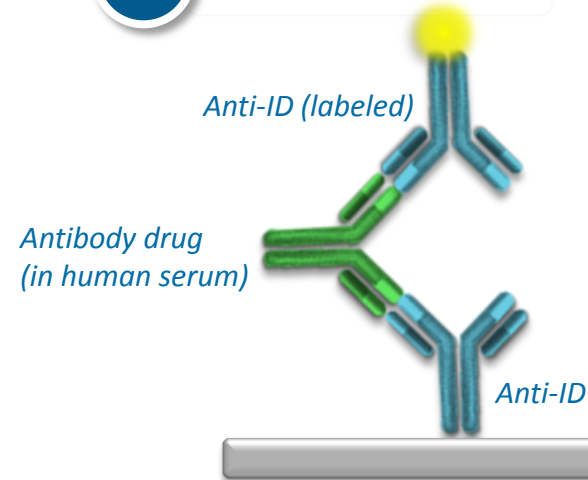
Typical PK Assay Formats



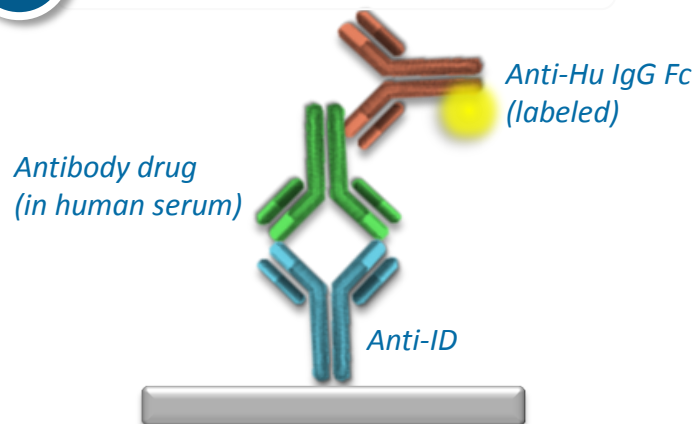
1 Antigen Capture



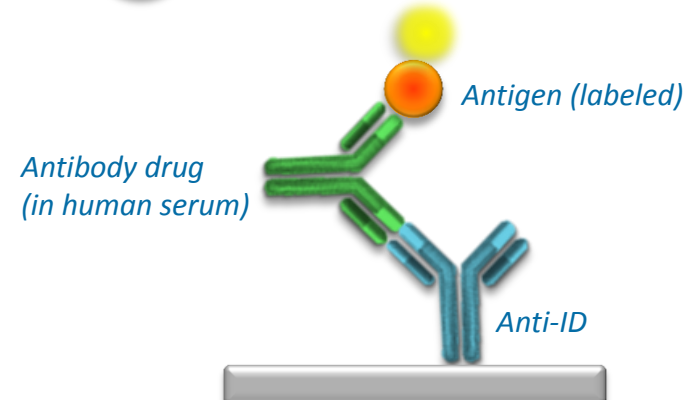
2 Anti-ID-Bridging



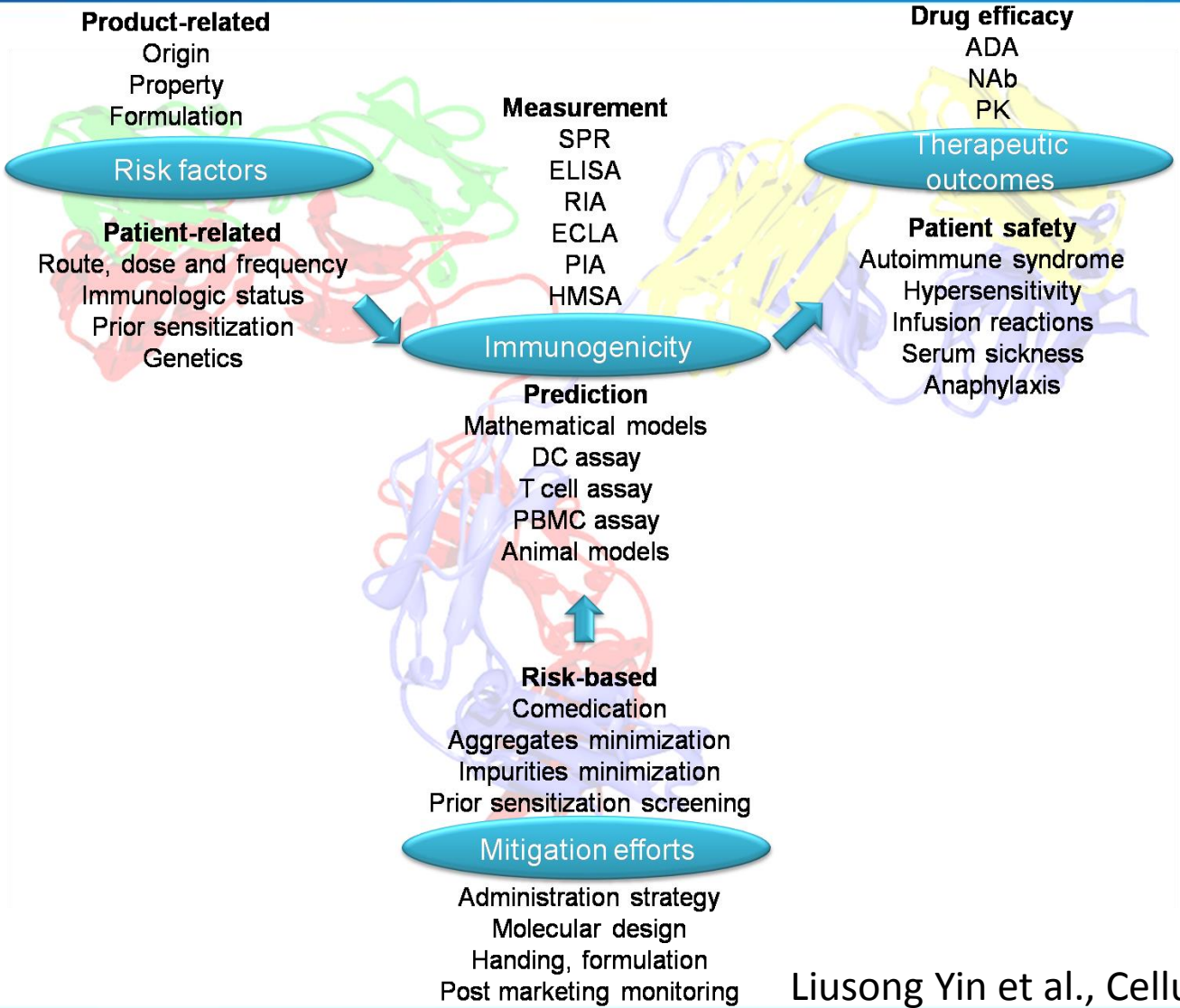
3 Anti-ID Capture Sandwich



4 Anti-ID-Antigen-Bridging



Immunogenicity of Therapeutic Antibodies

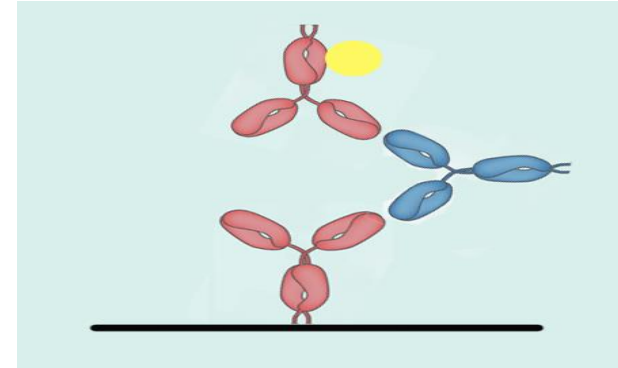


Liusong Yin et al., Cellular Immunol., 2015



❖ Select assay format

- Direct binding ELISA
- Bridging ELISA
- Radio-immunoprecipitation assay (RIPA)
- SPR
- Others



❖ Select assay reagents (FDA guideline)

Develop positive controls

- Immunize animals to generate positive controls;
- Purified antibodies can be spiked into the matrix selected for screening assay.
- **Rabbit** or **monkey** will be the recommended host animals.
- Detection of anti-idiotypic antibodies is the most important assay validation for immunogenicity.

Establish negative control with a pool of sera from 5-10 non-exposed individuals.

Immunogenicity of FDA Approved mAbs



Product	Form	Target	Indication	Company	Approval
OKT3 (86%)	Murine IgG2a K	CD3	Graft reject	Ortho Biotech	1986
ReoPro (6-44%)	Chimeric Fab	GP IIb/IIIaR	Angioplasty	Centocor	1994
Rituxan (1%, 3/356)	Chimeric IgG1 K	CD20	NHL	Genentech	1997
Zenapax (14-34%)	Humanized IgG1 K	CD25	Graft reject	Roche	1997
Remicade (10%)	Chimeric IgG1 K	TNF	RA,CD	Centocor	1998
Simulect (4/339, 2/133)	Chimeric IgG1 K	CD25	Graft reject	Novartis	1998
Herceptin (<1%, 1/903)	Humanized IgG1 K	HER-2/NEU	Breast cancer	Genentech	1998
Synagis (1-2%)	Humanized IgG1 K	RSV	RSV	Medimmune	1998
Mylotarg (0%, 0/277)	Humanized IgG4 K	CD33	AML	Wyeth	2000
Campath (2%, 4/211)	Humanized IgG1 K	CD52	CLL	ILEX	2001
Zevalin (4%, 8/211)	Murine IgG1 K	CD20	NHL	IDEC	2002
Xolair (<0.1%, 1/1723)	Humanized IgG1 K	IgE	Asthma	Genentech	2003
Bexxar (99%, 219/220)	Murine IgG2a K	CD20	NHL	Corixa	2003
Raptiva (6.3%, 67/1063)	Humanized IgG1	CD11a	Psoriasis	Genentech	2003
Erbix (5%, 28/530)	Chimeric IgG1 K	EGF-R	Colorectal cancer	Imclone	2004
Avastin (0/500)	Humanized IgG1 K	VEGF	Colorectal cancer	Genentech	2004
Tysabri (10%)	Humanized IgG4 K	A4-integrin	MS	Biogen/IDEC	2004
Vectibix (0.3-4%)	Human IgG2 K	EGF-R	Colorectal cancer	Amgen	2006
Humira (5%, 58/1062)	Human IgG1 K	TNF	RA	Abbott	2002

Case Study 1: Ab Drug Targeting Soluble Antigen



Aim	To develop therapeutic mabs
Target	A ~20 kDa cytokine
Feature	Native Select ™ ELISA to ensure all epitopes on target exposed in soluble form
Affinity	KD= 1.2×10^{-10} M

Core requirement from client:

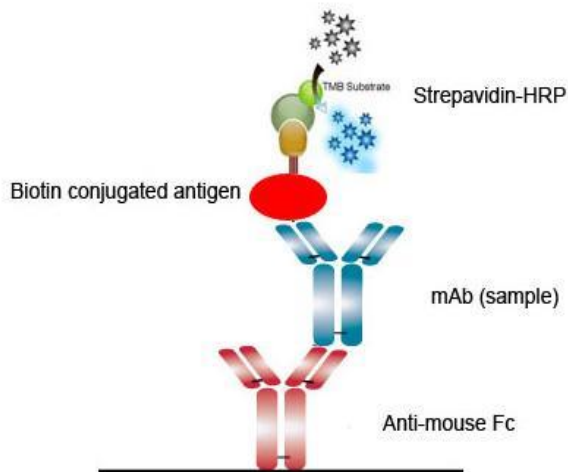
To develop functional mAbs recognizing target in circulation

Key to success:

Free the antigen presented in solution instead of coating on plate

Feedbacks:

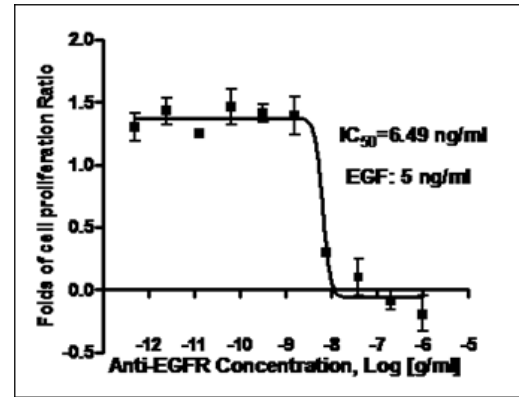
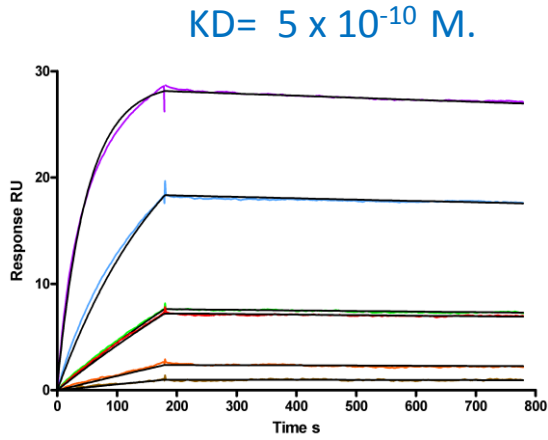
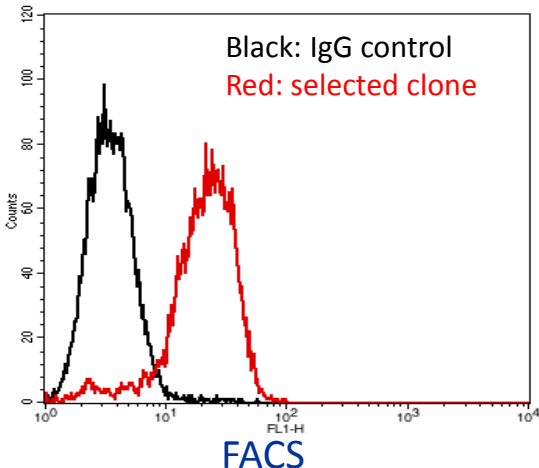
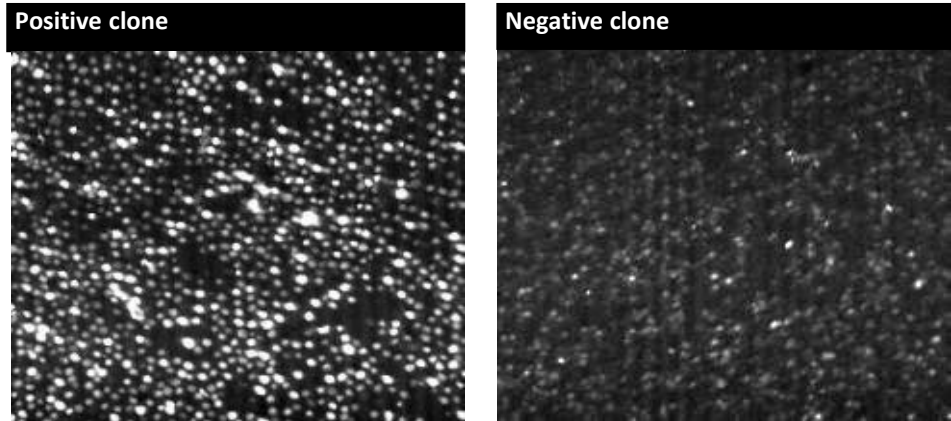
Delivered mAbs under optimization and prepared for downstream development



Case Study 2: Ab Drug Against Membrane-Bound Antigen

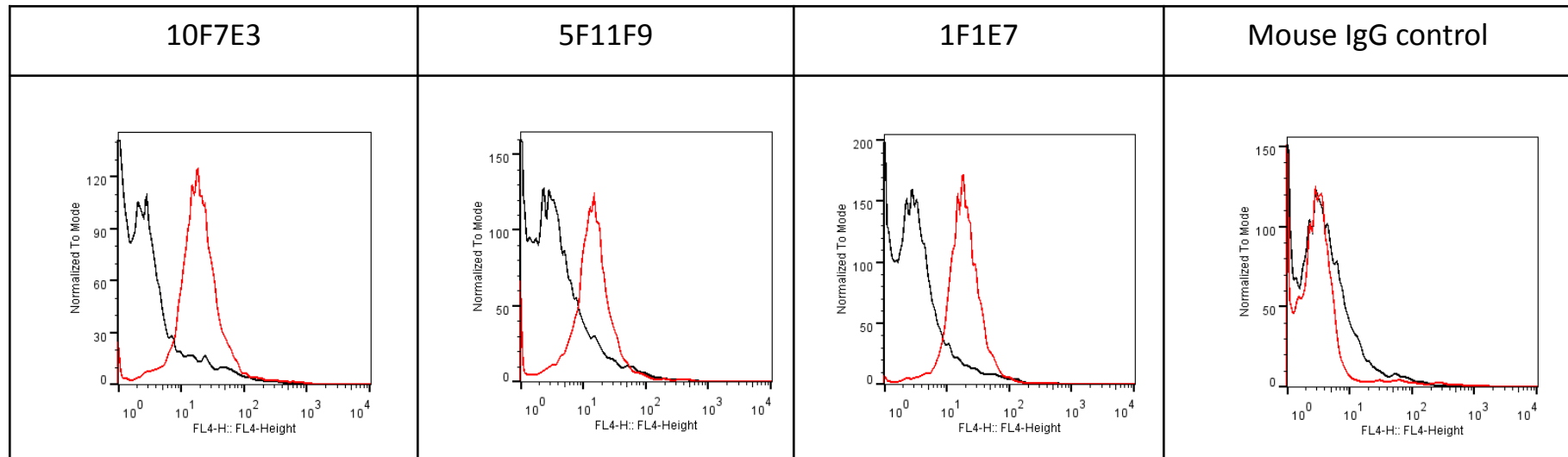


- Membrane-bound antigen – EGFR
- **HT March™** selected clone 5E10E9, which showed FACS positive;
- Functional assay proved the IC50 of 5E10E9 on EGF induced A431 cell proliferation is **42 pM**



Functional assay

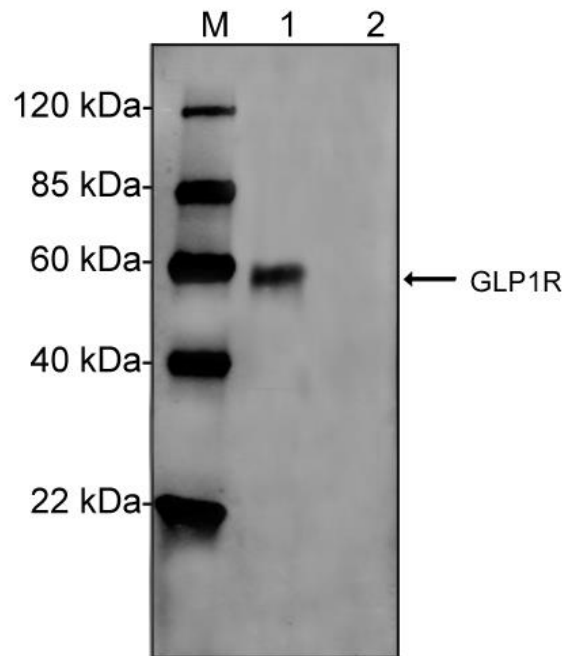
Case Study 3: Anti-GLP1R mAbs Generated by DNA Immunization



Flow cytometric analysis of CHO-K1/GLP1/G α 15 stable cells expressing GLP1R (GenScript, M00451) and CHO negative control cells with three mouse anti-GLP1R monoclonal antibodies (red and black respectively).

The signal was developed with iFluor647 conjugated goat anti-mouse IgG.

Case Study 3: DNA Immunization- Continued



Western blot analysis of virus like particle expressing GLP1R with **mouse anti-GLP1R (1F1E7) monoclonal antibody**.

Lane 1. GLP1R Antibody, mAb, Mouse (1 $\mu\text{g}/\text{ml}$)

Lane 2. Mouse IgG control (1 $\mu\text{g}/\text{ml}$)

Predicted size: 57 kDa

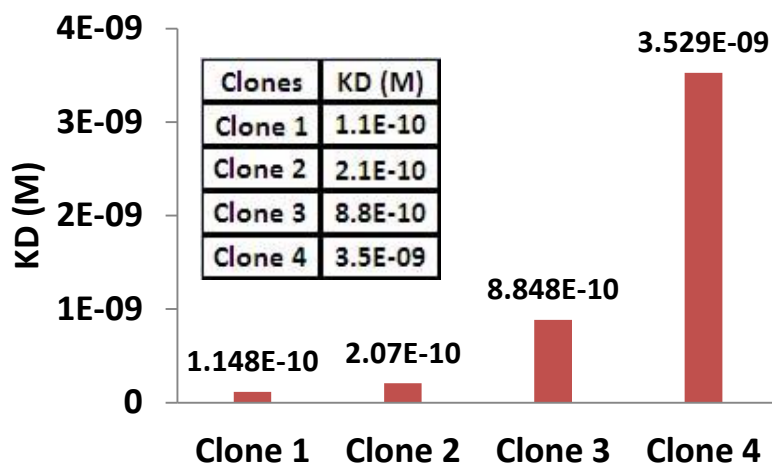
Observed size: 57 kDa

The signal was developed with IRDyeTM800 conjugated affinity purified goat anti-mouse IgG.

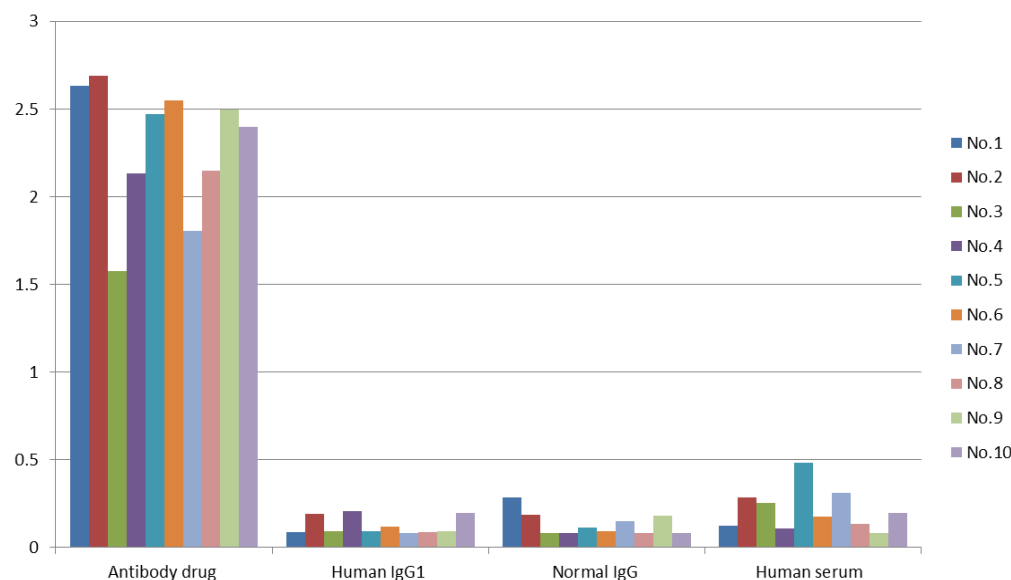
Case Study 4: Anti-Idiotypic Ab Generation



- Since 2009, GenScript has been specialized in making anti-idiotypic antibodies to support our customers' PK/PD and immunogenicity evaluations of antibody drugs. Our record of success rate is 100% with more than 100 projects.

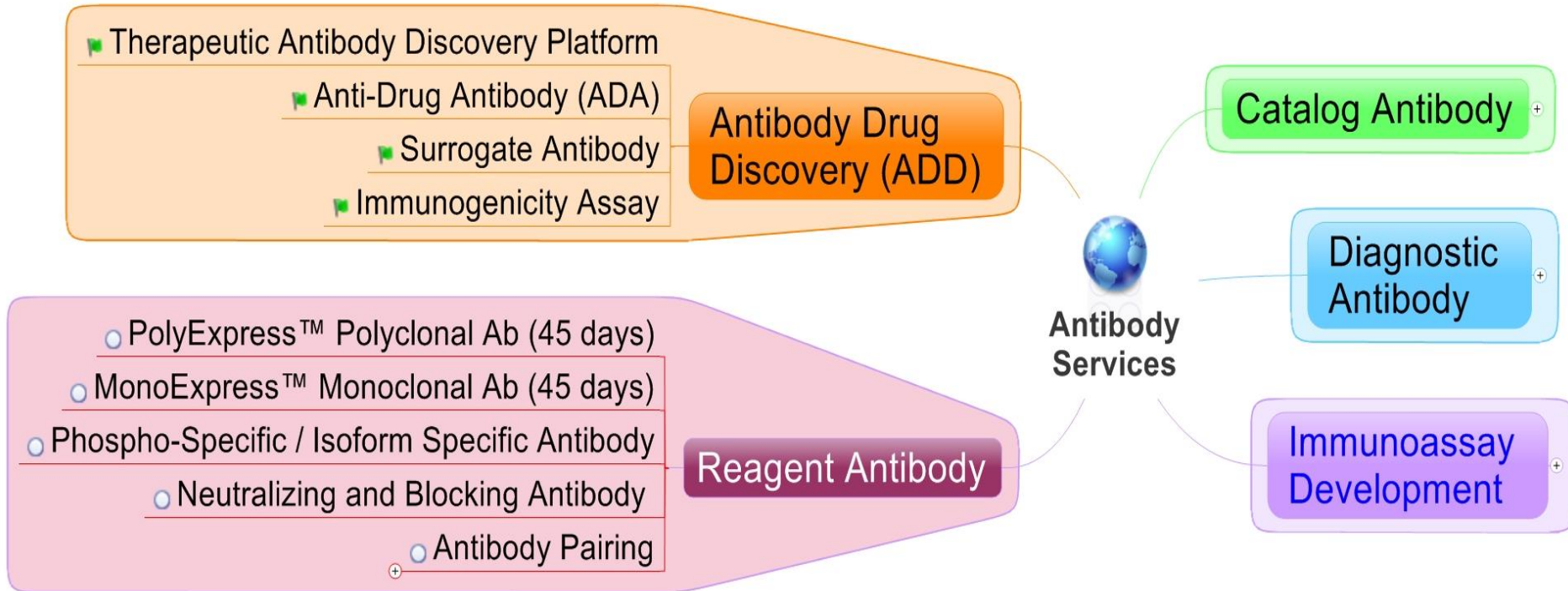


Anti-IDs with high affinity



Anti-IDs with high specificity

Scope of Antibody Services



Facility and Production Capacity



❖ AAALAC International and OLAW accredited animal facility

❖ 6,000 sq.m, hosting more than 8,000 rabbits and 12,000 rodents

❖ Providing a broad range of antibody services from individual cases to bulk orders

◆ High production capacity

Polyclonal Antibody Projects



500-600/Month

Monoclonal Antibody Projects



50-80/Month

Other Antibody Related Projects



50/Month



GenScript Ab Services Selection Guide



What's your application?

Antibody Drug Development?

Antibody antigen?

Anti-ID Ab service

Problematic antigen?

DNA Immunization Service

Need recombinant Ab?

HTP GtA

MamPower™

FragPower™

Gram Level Stable Cell Line

Reagent Antibody?

MonoExpress™

PolyExpress™




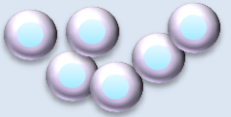




Phospho-specific Ab

Custom Ab Services

DNA Immunization Service Specifications



DNA Immunization Package Details* (Cat no. SC1693)

Step	Description	Specification	Timeline
1	 Gene Synthesis & Validation	<ul style="list-style-type: none"> Codon optimization Gene synthesis & plasmid preparation <i>In vitro</i> cell transfection for expression validation 	 2-3 weeks
2	 Immunization	<ul style="list-style-type: none"> DNA immunization via gold particle bombardment with gene gun Test bleed by ELISA 	8-10 weeks
3	Cell Fusion & Screening 	<ul style="list-style-type: none"> Animals selected for fusion based on titer Primary screening by whole cell based ELISA or FACS Customer can evaluate hybridoma supernatants and select the top clones for their application <i>Additional screening options available</i> 	 4-6 weeks
4	Subcloning, Expansion & cryopreservation 	<ul style="list-style-type: none"> Hybridomas are subcloned by limiting dilution according to the evaluation result from the customer, then expanded & frozen 	
5	 Monoclonal Antibody Production (optional)	<ul style="list-style-type: none"> Production of mAbs for each cell line with roller bottle culture Purification ELISA and/or FACS results 	 Based on project

Anti-idiotypic Antibody Service Specifications





Anti-Idiotypic Antibody Packages		
Services	Anti-idiotypic mAb	Anti-idiotypic pAb
Starting material	Target antibody drug 2-3 mg	Target antibody drug 20 mg or more
QC	Cross-reactivity with control IgG <10%	Cross-reactivity with control IgG <10%
Deliverables	Hybridoma cell lines, supernatants and purified anti-ID antibody (optional)	0.5-3 mg purified anti-ID antibody/rabbit

Features of GenScript Anti-ID Platform:

- High specificity and high affinity
- High speed production: **2-3 months**
- Antigen ligand blocking, epitope binning, and antibody pairing
- Proven track record: **100% success rate**
- **PK** and **IR assay** development

MonoExpress™ Service Specifications



Protein Antigen Packages	Guarantees	Starting Material	Immunization	Customer Screening	Deliverables	First Delivery
MonoExpress™ Premium SC1711 	<ul style="list-style-type: none"> Application-positive for one clone* ELISA ≥ 1:64,000 	<ul style="list-style-type: none"> Protein antigen produced by GenScript You provide: name, sequence (NCBI accession number if available) of target protein 	5 Balb/c mice or 3 rats	10 ELISA(+) supernatants for evaluation in client's own experimental system*	<ul style="list-style-type: none"> Up to 5 hybridoma cell lines of customer's choice* 2 mg purified mAb from one chosen hybridoma 200 µg immunogen protein 	11 weeks
MonoExpress™ Basic SC1717 	<ul style="list-style-type: none"> WB positive for immunogen ELISA ≥ 1:64,000 	We use your provided protein antigen as the immunogen	5 Balb/c mice or 3 rats	5 ELISA(+) supernatants for evaluation in client's own experimental system*	<ul style="list-style-type: none"> Up to 5 hybridoma cell lines of customer's choice* 2 mg purified mAb from one chosen hybridoma 	6-7 weeks

Peptide antigen packages also available: MonoExpress™ Gold (3 peptide antigens), Silver (2 peptide antigens), Bronze (1 peptide antigen).



◆ Antibody drug development

- **DNA Immunization** service offers a solution for Ab production against problematic antigens
- Premium HT hybridoma development and screening
- Recombinant antibody production services: HTP Gene to Ab, transient & stable rAb expression
- **Anti-idiotypic** antibody – an ideal tool for antibody drug PD/PK and immunogenicity studies.

◆ Reagent Ab - quality and reproducibility

- **MonoExpress™ Premium** application-guaranteed mAb production using protein antigen; guarantee that at least one clone will work in customers' own applications: Western Blot, IF/flow cytometry, IHC, IP/ChIP, Sandwich ELISA, functional assays, etc.
- **PolyExpress™ Premium** - Western Blot guaranteed pAb production services using protein antigen

About GenScript

