HUmanized Genomic Orthologs for Antibody development

Accelerating Antibody Discovery with Fully Human Antibody Mouse HUGO-Ab® and Single B Cell Screening Technology AbDrop®

------ Empower You for Breakthroughs in Antibody Drug Development !

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Cyagen and Biointron Partner to Empower Antibody Discovery

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HUGO-Ab®

Transgenic Mice for Human Antibody Discovery

The World's Leading Provider of

Custom Animal Models

- Custom Model Generation
- Ready-to-Use Catalog Mouse Models
- Drug Development Mouse Models
- Downstream Breeding & Cohorts
- Preclinical Drug Development CRO Services

BIOINTRON

AbDrop®

Droplet-Based Antibody Discovery Platform

The World's Leading Provider of

Antibody Discovery and Antibody Recombinant Production

- Antibody Production
- CHO-K1 Stable Cell Line
- VHH Antibody Discovery
- Single B Cell Screening
- Affinity Maturation
- Antibody Humanization
- Over Expression Cell Line
- Isotype Control/ Target Positive Antibody

Overview

1. Why is the transgenic human antibody mouse important for antibody drug development?
2. What are the advantages of human antibody mouse HUGO-Ab®?
3. How to accelerate antibody discovery with high-throughput single B technology AbDrob® in HUGO-Ab® mice

Trends and Distribution of Approved Antibody Drugs: An Overview

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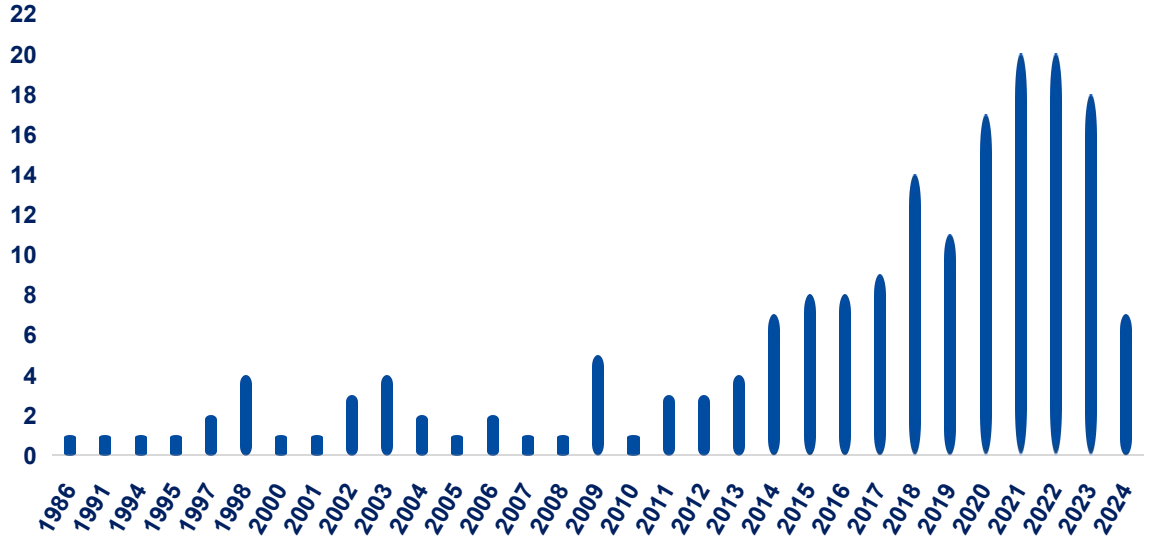
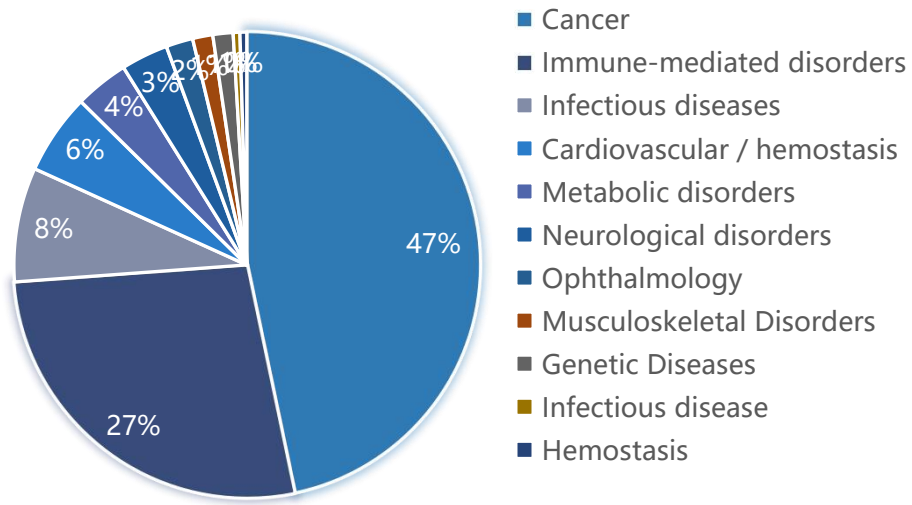
Approved Antibody Drugs by Years



Approved Antibody Drugs by Therapeutic Areas

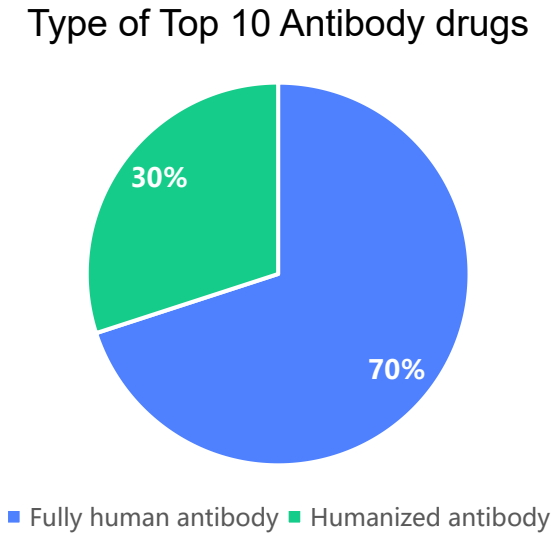


- The number of approved antibody drugs has increased significantly since the first approval in 1986 and has been a noticeable upward trend from 2015 onwards
- A substantial portion of the approved antibody drugs targets cancer and Immune-mediated disorders, making up 74% of the total approvals.
- High specificity, versatility and reduced side effect make antibody drugs a powerful option for treating a wide array of diseases, contributing to their increasing prevalence and success in clinical settings

Data collected from https://www.antibodysociety.org/

Fully Human Antibodies are Becoming a Significant Trend

Drug Name	Manufacturer(s)	Sales 2023 (USD M)	Diseases	Specification
Keytruda	Merck	25011	Cancer	Humanized antibody
Humira	AbbVie	14404	Autoimmmune	Fully human antibody
Dupixent	Sanofi	11590	Autoimmmune	Fully human antibody
Stelara	Johnson & Johnson	10858	Autoimmmune	Fully human antibody
Darzalex	Johnson & Johnson	9744	Cancer	Fully human antibody
Opdivo	Bristol-Myers Squibb	9009	Cancer	Fully human antibody
Skyrizi	AbbVie	7763	Autoimmmune	Humanized antibody
Ocrevus	Roche	5750	Autoimmmune	Humanized antibody
Cosentyx	Novartis	4980	Autoimmmune	Fully human antibody
Imfinzi	AstraZeneca	4237	Cancer	Fully human antibody



- Top 10 antibody drugs generated 100 billion US dollars of sales in 2023.
- 70% of the top 10 antibody drugs are fully human antibodies, indicating a dominant trend towards using human antibodies in antibody drug development.

Data collected from https://www.drugdiscoverytrends.com/

Usage of human antibody to overcome immunogenicity issues

First monoclonal antibody drug: Orthoclone OKT3, as a mouse-derived antibody, was withdrawn from the market in 2006 due to immunogenicity issues:

HAMA

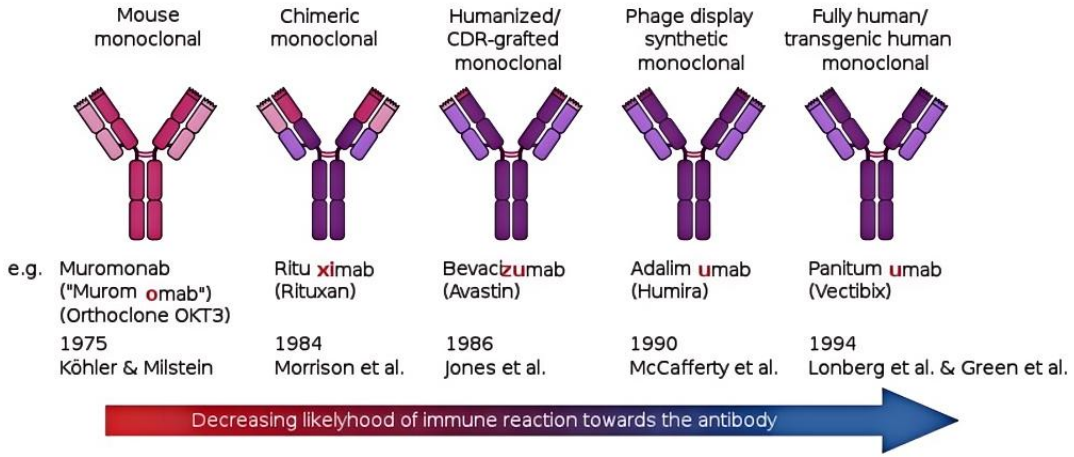
Recognized by human immune system, triggering production of human anti-mouse antibodies (HAMA)

High Dose

Mouse-derived antibodies have a short half-life, resulting in rapid clearance. Repeated high doses of drug further increase HAMA production

Allergy

In rare cases, the use of mouse-derived antibodies could lead to severe allergic reactions, and in some instances, it even resulted in the patient deaths



Format	Techniques	Advantages	Disadvantages	Immuno-genicity
Chimeric Antibody	Fusion of mouse variable antibody regions with human IgG	No	Not used anymore due to immnogenicity issues	High
Humanized Antibody	Grafting CDRs onto human frameworks	Lower cost, typical and traditional.	Risk of humanization level and success rate, high technical barrier	Moderate
Fully Human Antibody	Human phage display libraries	Good for toxic antigen	Light-Heavy chain not naturally paired	Low or moderate
	Single B cell selection from human PBMCs	Suitable for infectious diseases antigens	Limited to infectious diseases	Low
	Transgenic human antibody mice	**Suitable for most situations**	**Limited access to use for the high cost and complicated licensing terms**	**Low**

Advantages of Using Transgenic Human Antibody Mouse

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1. Lower Risk of Immunogenicity

- Naturally paired light and heavy chain
- Reducing the risk of immunogenicity and adverse reactions in patients

2. Efficient Sceening of High Specificity, High Affinity, Functional, and Developable Antibodies

- Compatible with hybridoma, phage or yeast display, and single B cell screening without limitations
- Natural molecules derived from in vivo evolution and selection

3. Time Saving

- Antibody humanization engineering is not required and 3-4 months are saved
- No risk of loss of affinity and developability

HUGO-Ab® Fully Human Antibody Mouse

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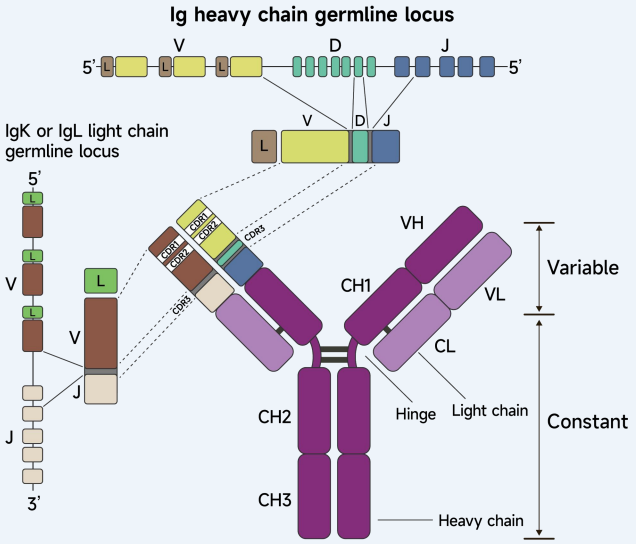
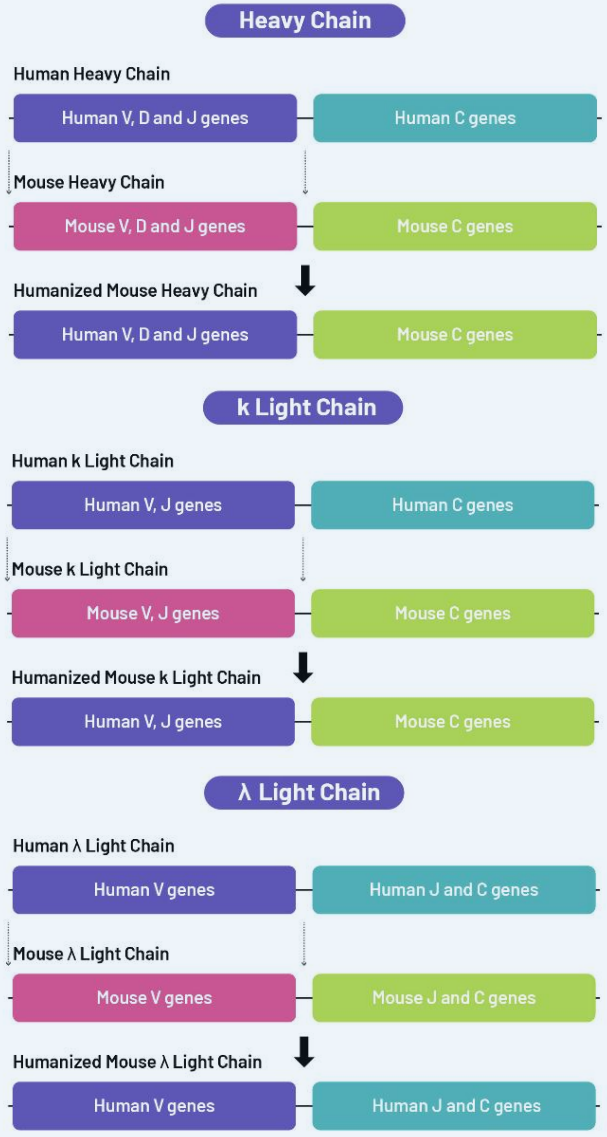




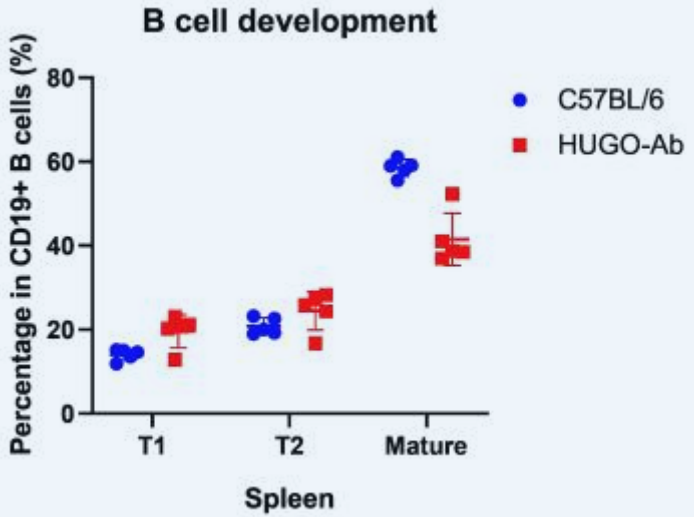
HUGO-Ab®

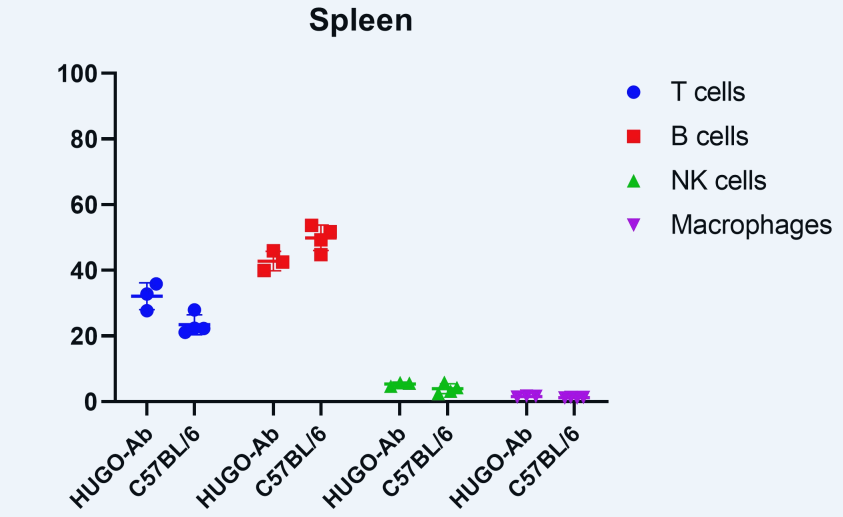
▸ **HU**manized **G**enomic **O**rtholog for **Ab**ody development

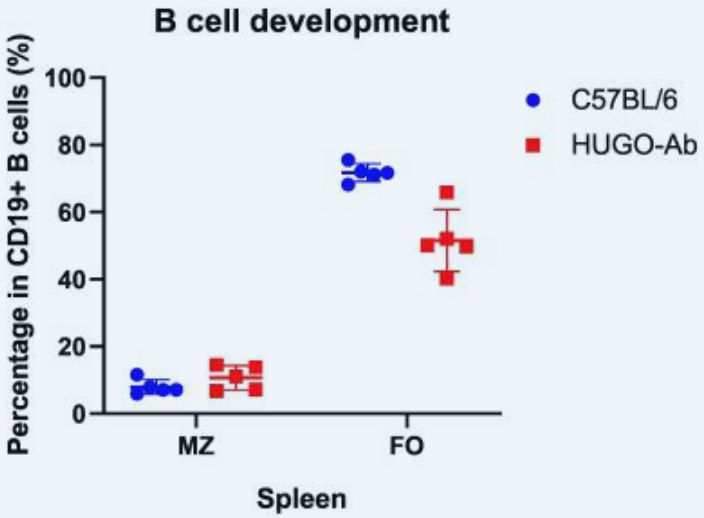
- Developed based on Cyagen's proprietary patent TurboKnockout® ES targeting technology
- In situ replacement of the full-length sequences of human VH, Vκ, and Vλ
- Available in C57BL/6, BALB/c, and SJL strains

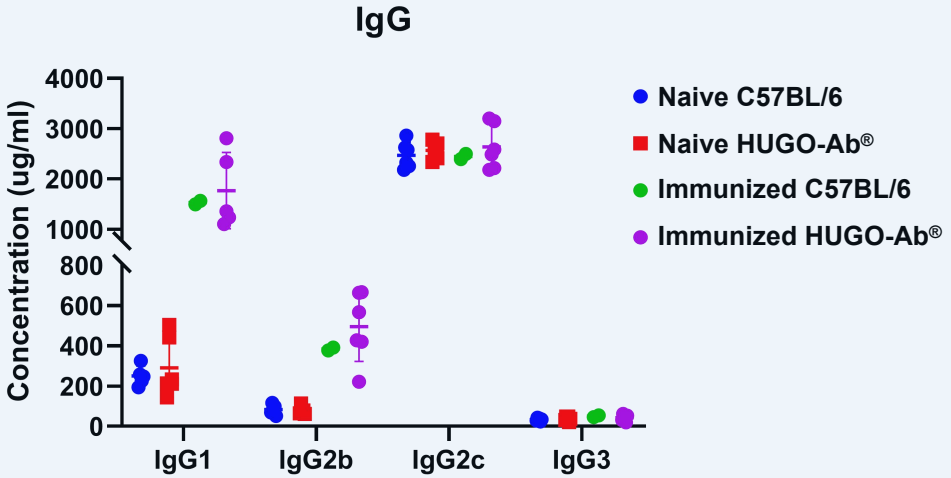
Ready to use with no string attached

HUGO-Ab® B cell development



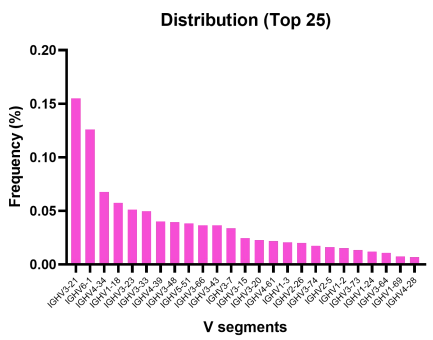




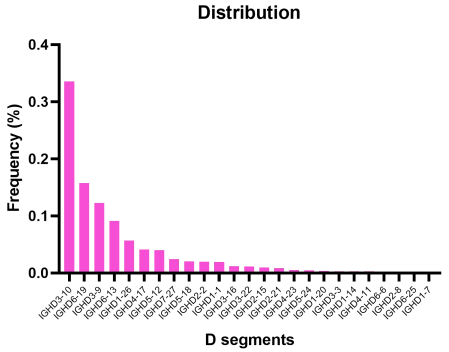


- HUGO-Ab® demonstrates normal B cell development compared to wild-type C57BL/6 mice
- HUGO-Ab® shows expected immunoglobulin IgG levels similar to those of wild-type C57BL/6 mice.

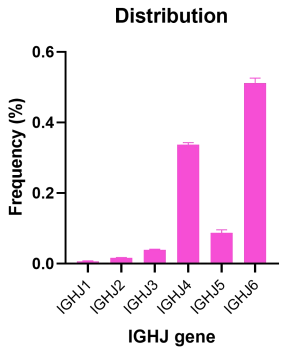
HUGO-Ab® Heavy Chain VDJ Rearrangement in Splenic B Cells



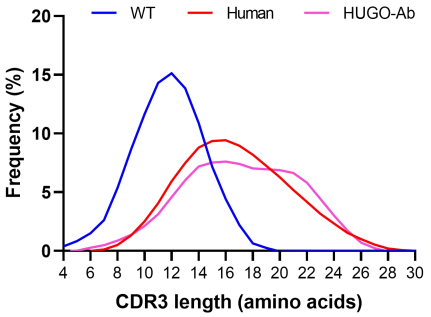
Distribution of V Gene Usage Frequency



Distribution of D Gene Usage Frequency

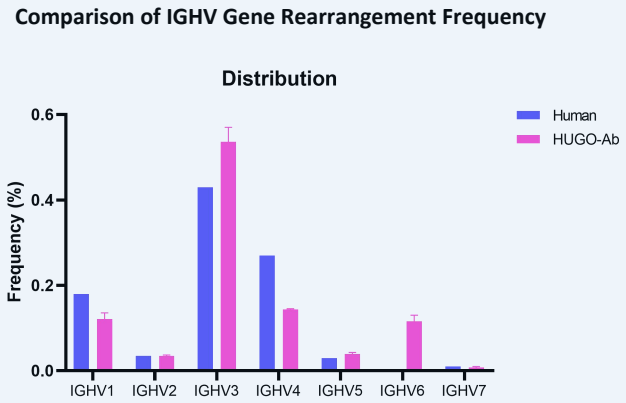


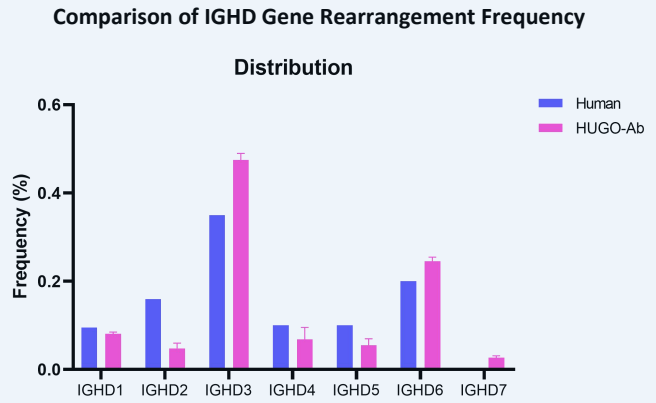
Distribution of J Gene Usage Frequency

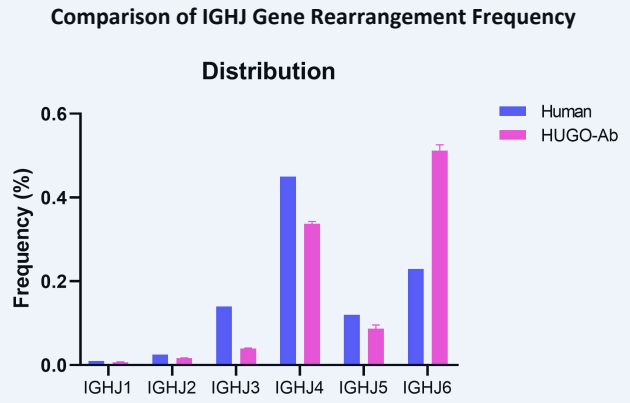


Distribution of CDR3 Length Frequency

Comparison of Gene Rearrangement Frequency

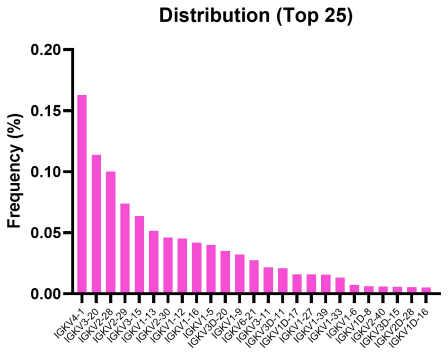




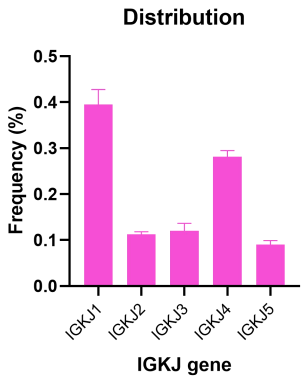


HUGO-Ab® Light Chain V(J) Rearrangement in Splenic B Cells

κ Light Chain Gene Rearrangement

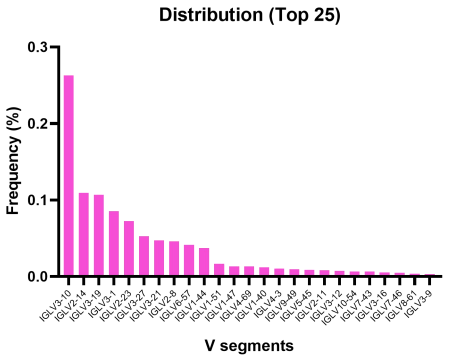


Distribution of V Gene Usage Frequency

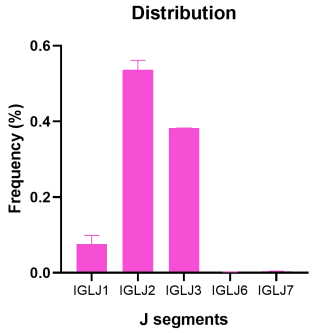


Distribution of J Gene Usage Frequency

λ Light Chain Gene Rearrangement

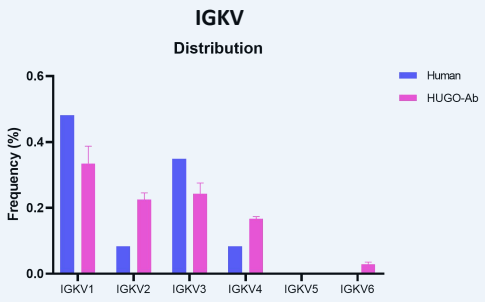


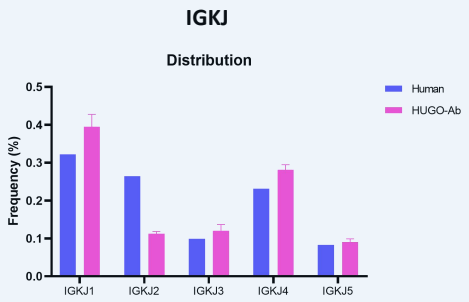
Distribution of V Gene Usage Frequency

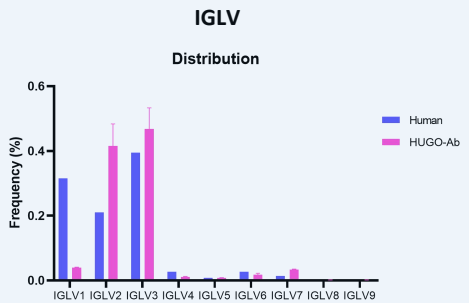


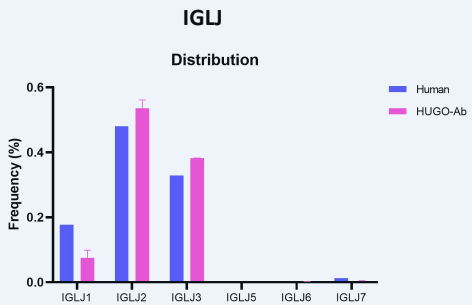
Distribution of J Gene Usage Frequency

Comparison of Gene Rearrangement Frequency

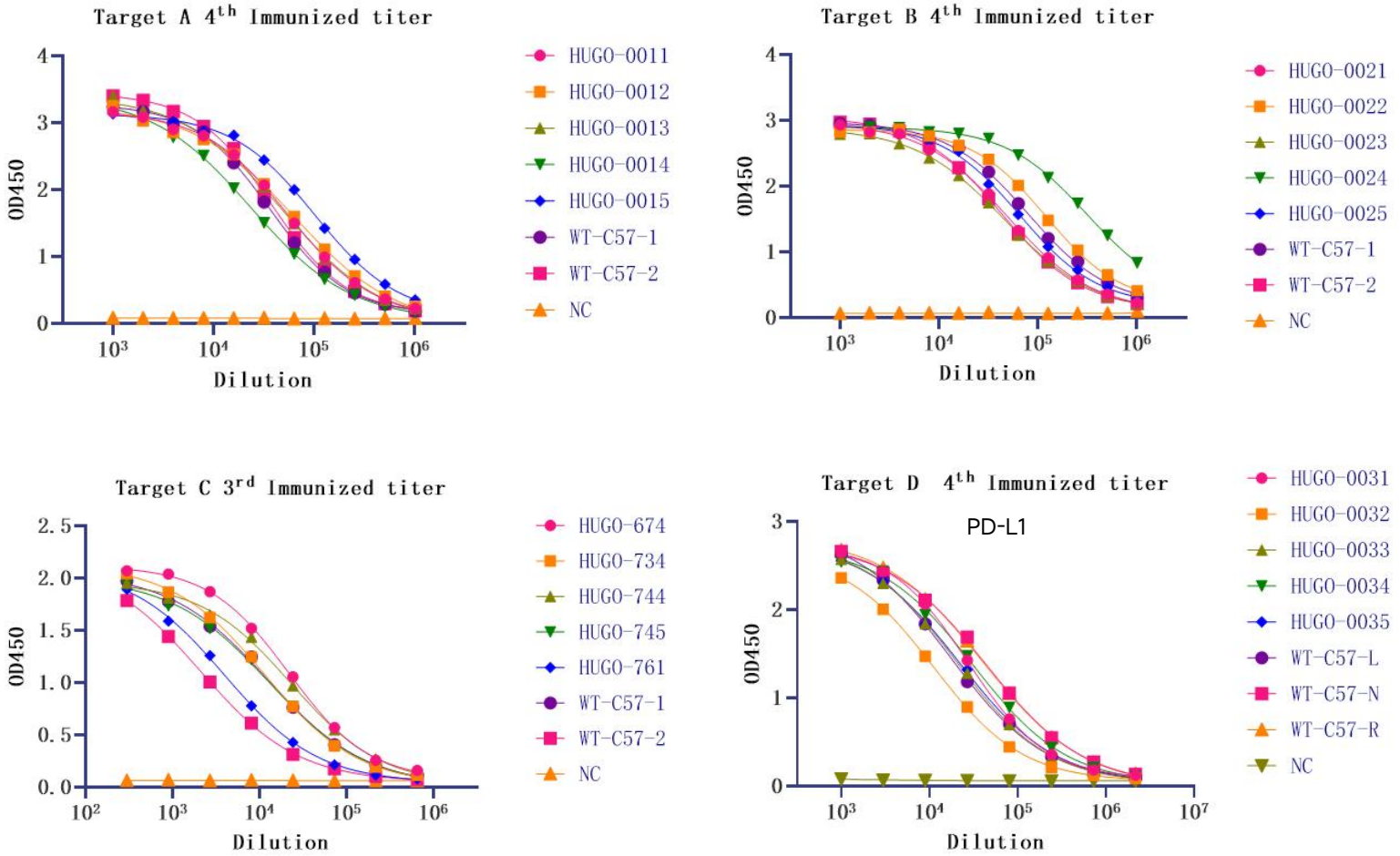








HUGO-Ab® Exhibit Excellent Immune Response Capabilities



- The serum antibody titer exceeds a dilution factor of half a million
- HUGO-Ab® exhibits a slightly stronger immune response compared to the wild type C57BL/6

Immunization Protocol

Stages	Immunization method
1st immunization (D0)	protein + CFA, SC
2nd immunization (D14)	protein + IFA, SC
3rd immunization (D28)	protein + IFA, SC
4th immunization (D43)	protein + IFA, SC
Boosting (D57)	protein, IP
Three days after boosting	Collect B cells

HUGO-Ab® Produce High-Affinity Antibody Molecules

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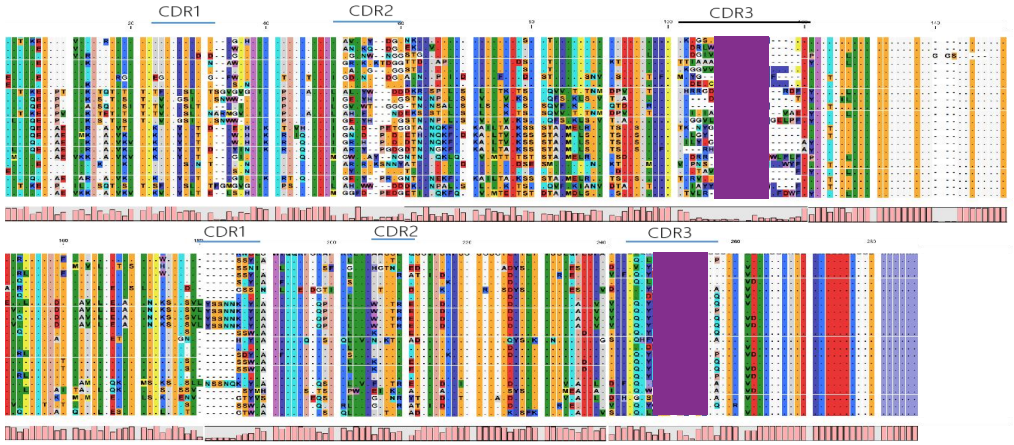
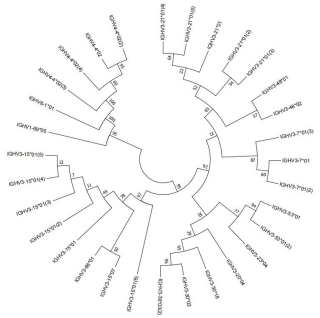
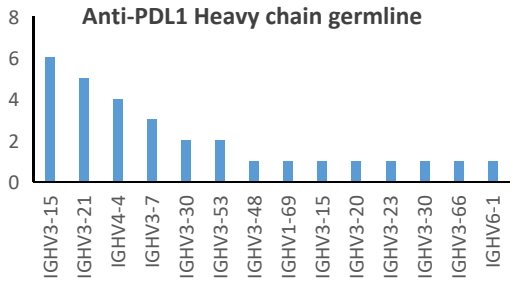
Full human antibodies against PD-L1

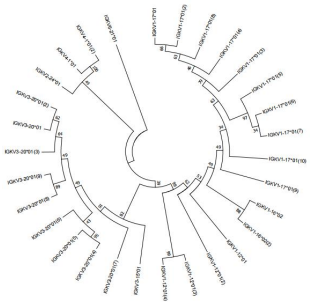


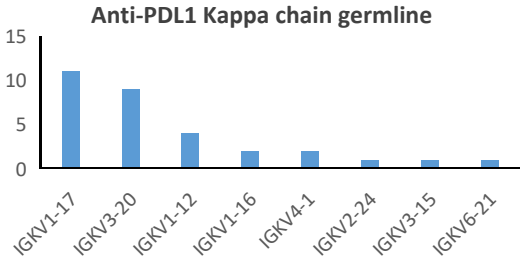
HC





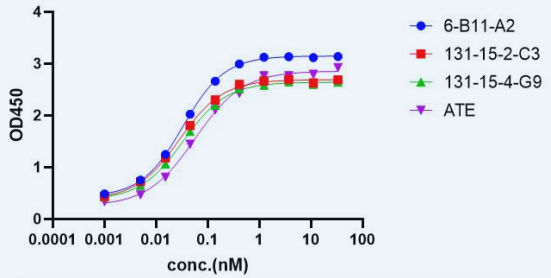
LC





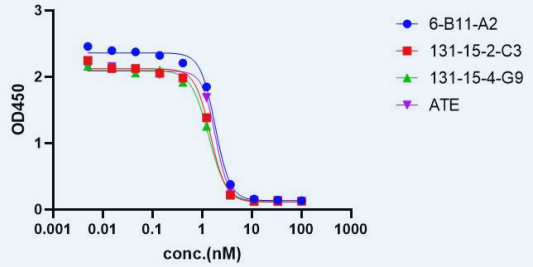
Anti-PDL1 affinity test

Anti-PDL1 binding to PD-L1 His affinity assay



	6-B11-A2	131-15-2-C3	131-15-4-G9	ATE
EC50	0.03304	0.02829	0.03357	0.05705

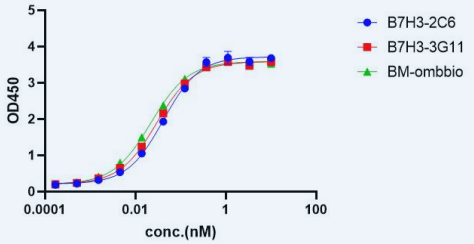
Anti-PDL1 blocking to PD1-mFc affinity assay



	6-B11-A2	131-15-2-C3	131-15-4-G9	ATE
EC50	1.839	1.474	1.354	1.842

Anti-B7H3 affinity test

Anti-B7H3 binding to B7H3-His affinity assay



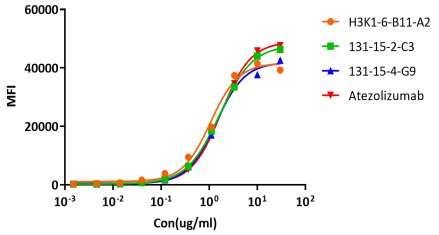
	B7H3-2C6	B7H3-3G11	BM-ombbio
EC50	0.04119	0.02962	0.02187

- Generating a diverse array of fully human VH and VL sequences.
- Generating high-affinity antibody molecules against antigens.

Human Anti-PD-L1 Antibodies Show Promising Affinity and Functionality

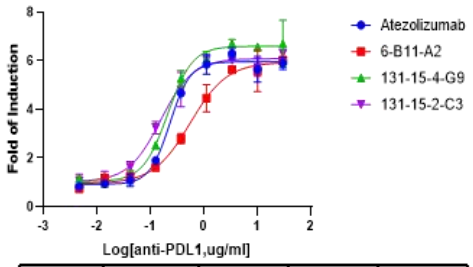
Cell-based assay

Anti-PDL1 binding to CHO-hPDL1 cell



	H3K1-6-B11-A2	131-15-2-C3	131-15-4-G9	Atezolizumab
EC50	1.064	1.659	1.387	1.776

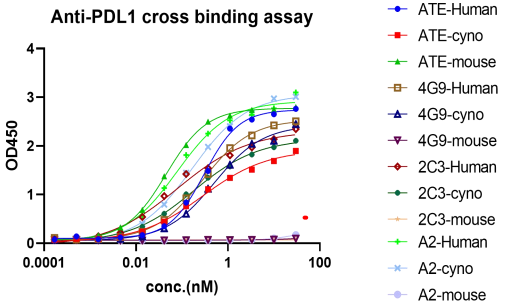
Anti-PDL1 reporter cell blocking assay



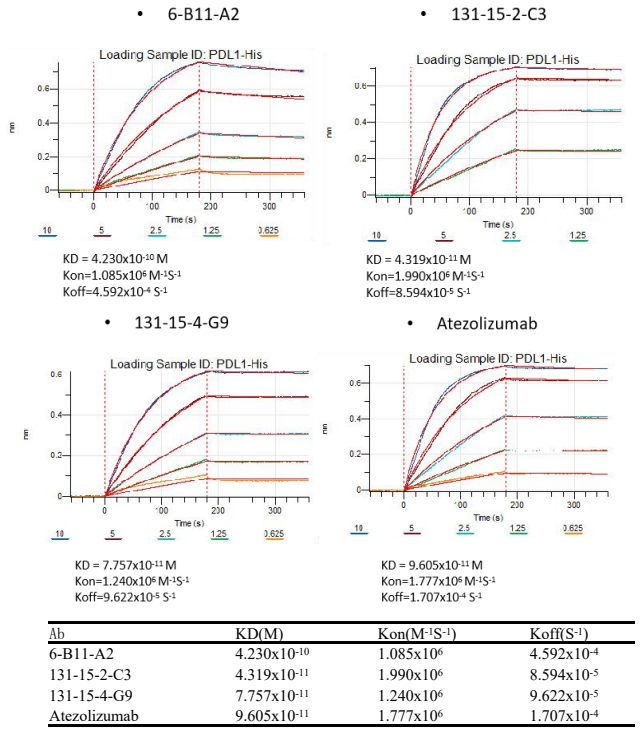
Ab	Atezolizumab	6-B11-A2	131-15-4-G9	131-15-2-C3
C50（ug/ml）	0.2519	0.5576	0.2053	0.1419

Cross binding assay

Anti-PDL1 cross binding assay



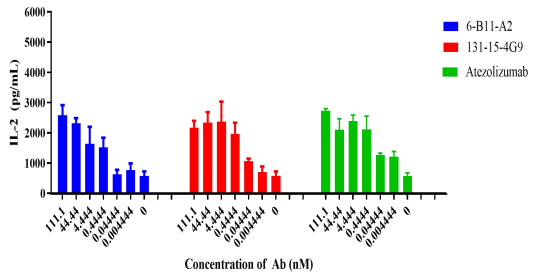
Kinetic test (BLI)



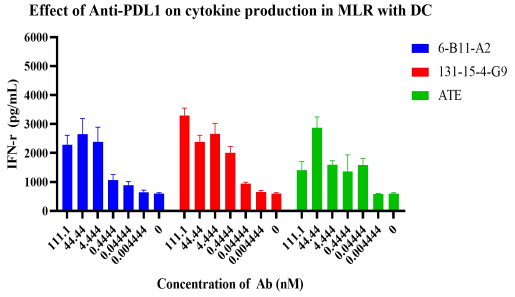
Ab	KD(M)	Kon($M^{-1}S^{-1}$)	Koff(S^{-1})
6-B11-A2	$4.230x10^{-10}$	$1.085x10^{6}$	$4.592x10^{-4}$
131-15-2-C3	$4.319x10^{-11}$	$1.990x10^{6}$	$8.594x10^{-5}$
131-15-4-G9	$7.757x10^{-11}$	$1.240x10^{6}$	$9.622x10^{-5}$
Atezolizumab	$9.605x10^{-11}$	$1.777x10^{6}$	$1.707x10^{-4}$

MLR assay

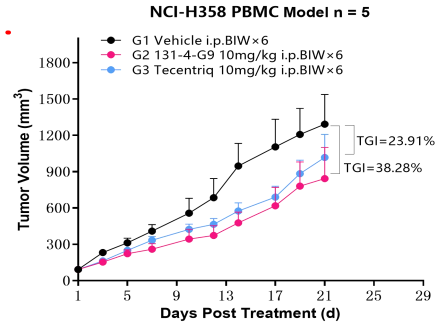
Effect of Anti-PDL1 on cytokine production in MLR with allogeneic DC



Effect of Anti-PDL1 on cytokine production in MLR with DC



In Vivo assay



- The anti-PD-L1 candidate molecule G9 is isolated from Hugo-Ab mice and demonstrates enhanced affinity and biological activity compared to atezolizumab

HUGO-Ab® Possesses Rich Antibody Diversity

Product	Competitor1	Competitor2	Competitor3	Competitor4	Competitor5	Competitor6	HUGO-Ab
Method	**In situ ES targeting**	**In situ ES targeting**	Transgenic	Transgenic	Transgenic	Not disclosed	**In situ ES targeting**
Parental strain	BALB/c, CD1, NOD	C57B/L6	C57B/L6,FVB, 129	SD/HSD	C57B/L6, SJL	C57B/L6	**C57B/L6, BALB/c, SJL**
HC Fc	**Mouse IgG1,2b,2c,3**	**Mouse IgG1,2b,2c,3**	Rat IgG1,2b,2c	Rat IgG1,2a,2b	Rat IgG1,2a,2b	Not disclosed	**Mouse IgG1,2b,2c,3**
HC Repertoire	**Complete**	**Complete**	Partial	**Complete**	**Complete**	**Complete**	**Complete**
κ LC Repertoire	Partial	**Complete**	Partial	Partial	Partial	Partial	**Complete**
λ LC Repertoire	Partial	No	Partial	Partial	Partial	Partial	**Complete**

HUGO-Ab®

▸ **HU**manized **G**enomic **O**rtholog for **Ab**ody development

- Engineered using Cyagen's TurboKnockout® ES technology
- In situ replacing VH, VK and VL
- Available in C57BL/6, BALB/c, and SJL strains
- Complete sequences of human-derived VH, Vκ and Vλ genes

Different Technologies for Antibody Discovery

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- Single B cell technology combines the advantages of hybridoma and phage display technologies

Technology	Diversity	Average Affinity	Species	Binder	Time
Hybridoma	✔	✔✔✔	Mouse	Natural form of IgG Chromosome instability	🕒🕒🕒
Phage Display	✔✔	Depends	Diverse library	Unpaired light and heavy chains	🕒🕒
Single B Cell technology	✔✔✔	✔✔✔	Mouse/ Rabbit	Paired light and heavy chains	🕒

AbDrop®: Droplet Microfluidics Single B-cell Technology

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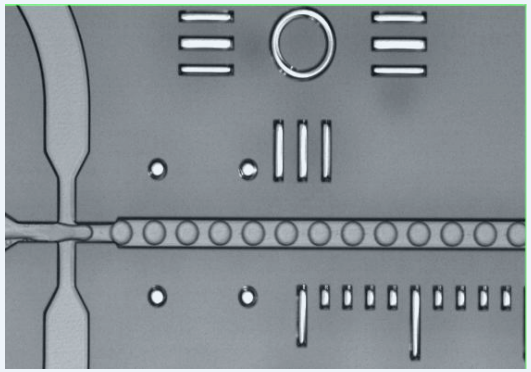
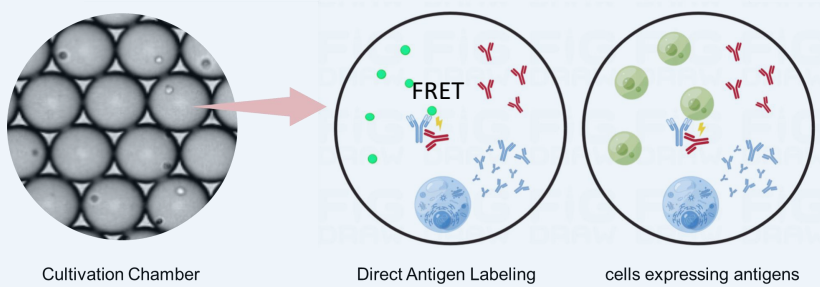
Macro-droplet generation

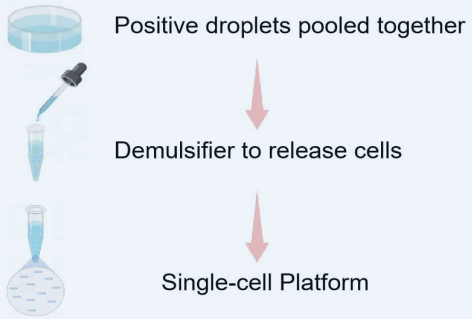


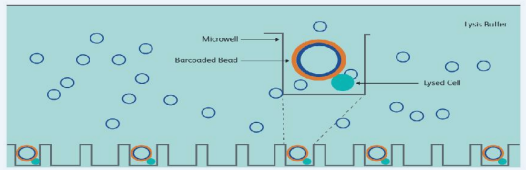
Detection system for protein based binder or cell based binder selection

$$\text{Moles dye per mole protein} = \frac{A_{dye} \times \text{dilution factor}}{\varepsilon_{dye} \times \text{protein concentration (M)}}$$

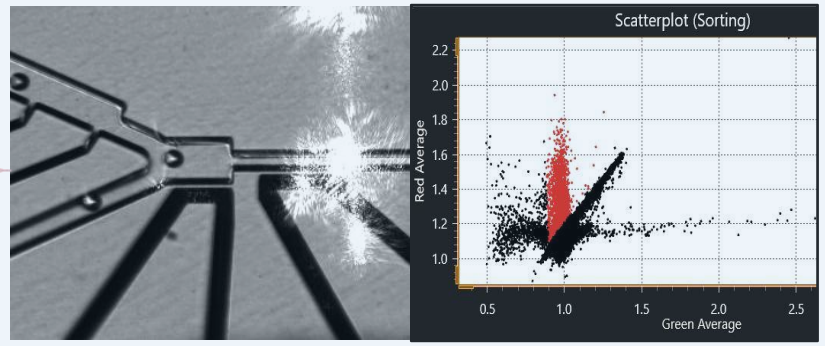


Exporting cells for 10X sequencing





Droplet sorting



BIOINTRON

AbDrop®

Droplet-Based Single B-cell Screening platform

- Screening millions of plasma B cells in one day
- NGS sequencing of single B cell in one week
- Obtaining hundreds of naturally paired heavy and light chain at one time
- From screening to candidates in one month

Advantages of AbDrop® technology

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Single B cell platform	Throughput	Automation	Cost	Sensitivity	Assay Capability	
					Multiplex testing	Capability on cell-based assay
Microengraved micro/nanowells-based	Medium (100,000/chip)	Low	Low	High	N	N
FACS-Based	High (millions cells/time)	Low	Low	Low	Y	N
Microfluidic Chamber-based (Beacon)	Relatively low (11,000/chip)	High	High	High	Y	Y
Microdroplets-based (AbDrop)	High (millions cells/chip)	Medium	Low	High	Y	Y

- AbDrop® has advantages compared to other technologies overall

Case Study 1: Efficient Screening of Antibodies Targeting Z Protein

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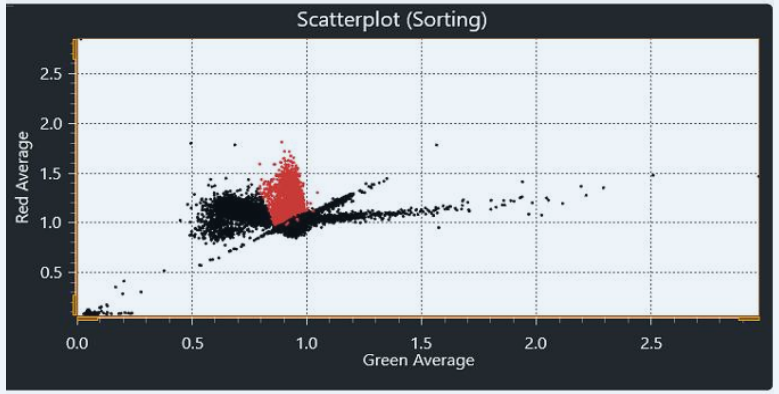
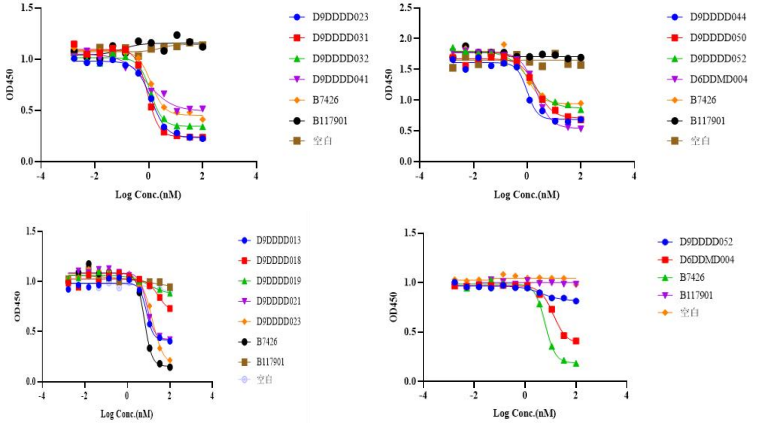
Basic info

Mouse	SBC1002-#1
Antigen	Recombinant Z protein
Titer	>64000
Number of cells	3.00E+08
Number of plasma cells	1.20E+06
Number of expression sequences	60

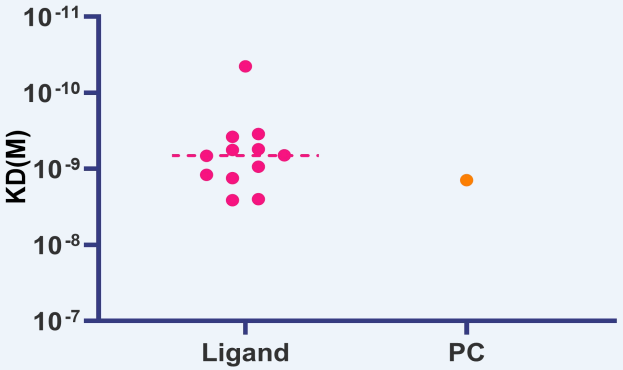
Sorting of positive microdroplet



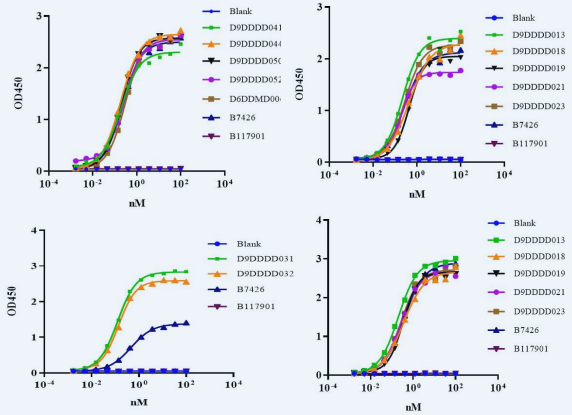
Blocking assay



Affinity measurement



HTP expression and ELISA assay



BIOINTRON

AbDrop®

- 306 antibody sequences were obtained
- 60 antibody sequences were expressed
- 42 antibody sequences out of 60 were validated and exhibited protein-binding activity
- 26 antibody sequences demonstrated blocking functionality
- 43% of expressed antibodies were high-affinity and functional

Case Study 2: HUGO-Ab® Shows Promising Traits Comparable to Wild-Type Mice

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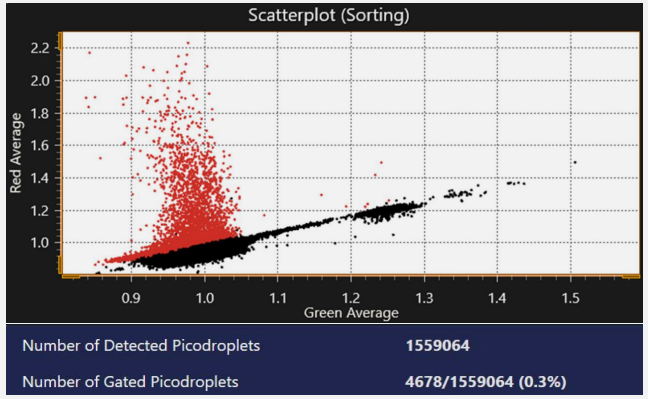
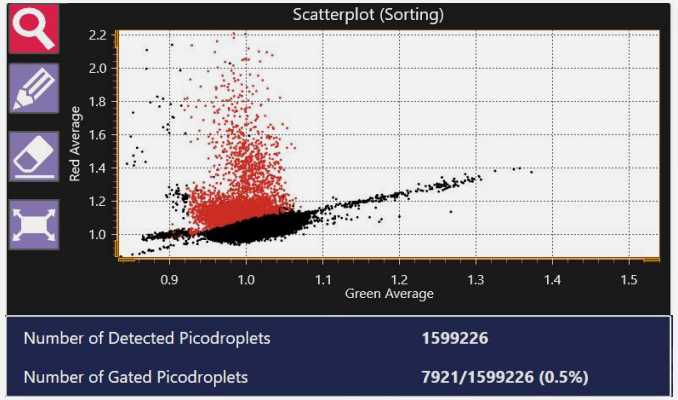
Anti-PD-L1 antibody screening in HUGO-Ab® and C57BL/6

WT positive droplet sorting



HUGO-Ab® positive droplet sorting

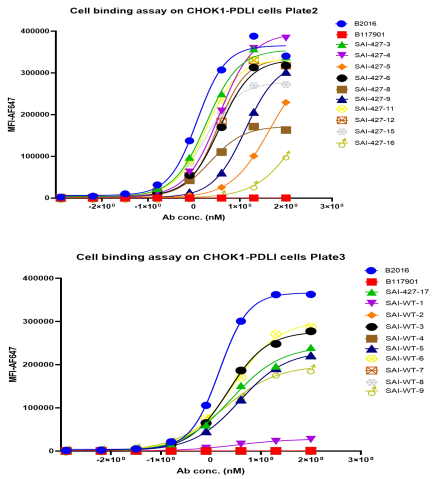


The total number of plasma cells enriched using CD138 beads: After 2 hours of culture, by gating positive cells, WT obtained 4,678 positive droplets, and HUGO-Ab® obtained 7,921 positive droplets.

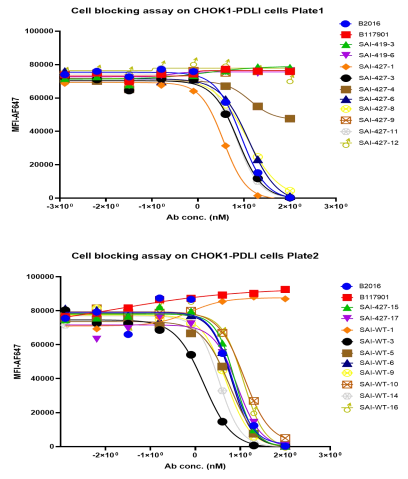
10x sequencing results

Mouse	Sorted droplets	Cell amount	Paired sequence	Unique sequence	Highest frequency
HUGO-Ab®	7917	1809	1466	413	56
WT	4706	1689	1248	142	174

Cell binding assay



Cell blocking assay



BIOINTRON **AbDrop®**

- 16 VS 17 antibody sequences show good affinity in HUGO-Ab® VS C57BL/6
- 6 VS 6 candidate molecules from HUGO-Ab® VS C57BL/6 surpassed the benchmark in binding and blocking activity
- Efficient screening of High affinity and functional antibodies in HUGO-Ab® combined with AbDrop®

Case Study 3: AbDrop® Demonstrates Advantages Over Hybridoma Technology

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Project summary

Antigen: **Protein X**

Homology: **90% to mouse protein**

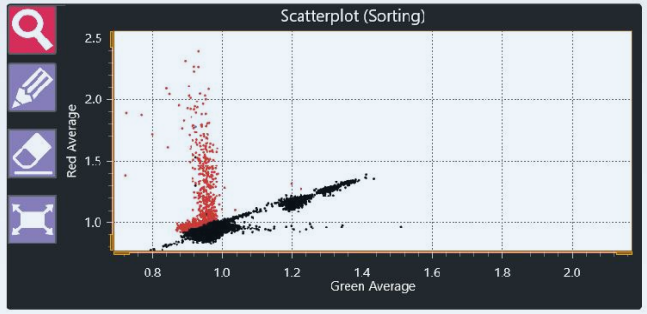
Immunization titer: **around 100K**

Hybridoma screening: **2 binders**

Microdroplets single B screening: **28 binders**

Microdroplet Enrichment

Total Sorted Picodroplets	957
Total Dispensed Picodroplets	0
Total Dispensed Plates	0
Dispensing Gate Ratio	NaN

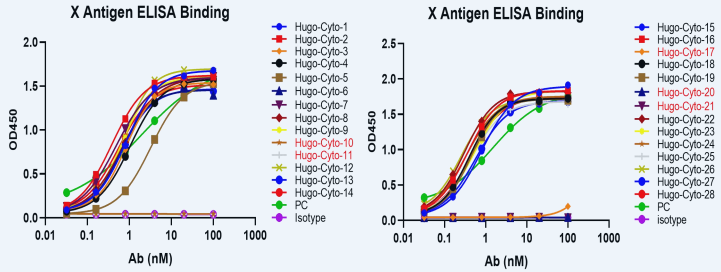


Sequencing Data Analysis

590	12,648	159
Estimated Number of Cells	Mean Reads per Cell	Number of Cells With Productive V-J Spanning Pair

Parameters	Account
Total Pairs	158
Unique Pairs	34
Highest frequency	170 (85)
Expression Pairs	34

Antibody Expression and Binding Validation



BIOINTRON

AbDrop®

- Target antigen 90% to mouse protein
- 2 binders were only obtained by hybridoma screening in HUGO-Ab®
- 28 binders were successfuly obtained from HUGO-Ab® combined with AbDrop®.

Advantages of HUGO-Ab® combined with AbDrop®

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1

Human Antibody Directly

Fully human antibody VH, VK and VL enable the mouse to generate human antibody molecules in highly rich diversity

2

No String Attached

Customers can easily access and use HUGO-Ab® mice without any milestone or royalty licensing fees.

3

Efficient and powerful

Millions of B cells are screened in a high-thoughput way, and binding and activity assays are encapsulated to maximize the number of potential candidates.

4

Fast timeline

Antibody screening can be completed in just one week, reducing the entire antibody discovery process from 6 months to 3 months

LIVE WEBINAR

Accelerating Antibody Drug Discovery with
Fully Human Antibody Mouse HUGO-Ab and
High-Throughput Single B Cell Screening

Date and Time : *July 25th, 2024 11:00am EST*

Save My Seat

Email: animal-service@cyagen.com
Email: info@biointron.com

Speaker: **Shun Zhou, Ph.D.**

Thanks