

# Overview of the CRO Service Platforms for Recombinant Protein Expression and Antibody Development

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Sino Biological Inc.

# Global Presence

30+ Global Distributors  
Customers in 90+ countries



**Branch in Philadelphia,  
USA**



**Branch in Frankfurt, Germany**  
**Local sales and service team with  
protein inventory**



**Headquarters in  
Beijing, China**



# Primary Facility



## Our people

~400 Employees & growing  
30% of staff with masters or PhD Degree

66,000 sq ft lab & office space  
25,000 sq ft GMP facility



**Certified by:**  
**ISO9001, ISO13485, CNAS**

# Core Business



## Products:

Recombinant proteins  
Antibodies  
Genes  
Cell culture supporting reagents

## Services:

Recombinant expression  
Antibody development  
Biological assays

# Recombinant Proteins

## Drug Targets (1500+)

- Support multiple therapeutic areas
- High purity and activity
- Multiple research animal models covered
- Hot targets: B7 Family, TNF Superfamily

## Cytokines & Receptors (1100+)

- Cover all cytokine families
- Over 12 species
- Popular targets: VEGF/TNF $\alpha$ /HGF/EGF/IL6/BMP2
- Offer GMP-grade

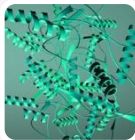
## Fc Receptors (100+)

- Largest Supplier for Fc Receptor Proteins
- Multiple species & Validated activity
- Human variants for IgG binding
- Hot: CD64/CD32a/CD32b/CD16/CD16a/FCGRT&B2M

## Virus Proteins (500+)

- Largest supplier worldwide
- Various types: Influenza, CoV, RSV, HIV, Ebola...
- Latest strains & vaccine strains
- For antibody drugs and vaccine development

## Featured CRO Services



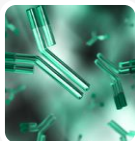
### Recombinant Protein Production Services

- Mammalian Cells, Insect Cells, *E. coli* Available



### Recombinant Antibody Production Services

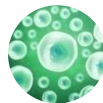
- High-throughput Antibody Production Service
- Large-scale Antibody Production Service



### Antibody Development Services

- Fast Mouse mAb Development
- Rabbit mAb Development
- Fast Rabbit pAb Development
- Anti-idiotypic Antibody Development

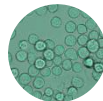
## Recombinant Production Platforms



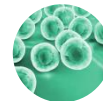
HEK293/CHO  
Transient system



*E. coli* System



Baculovirus-insect  
System



CHO/HEK293 Stable Cell  
Line Development

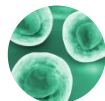
## Antibody Development Platforms



Mouse mAb Development  
(Hybridoma)



Rabbit mAb Development  
(Phage Display)

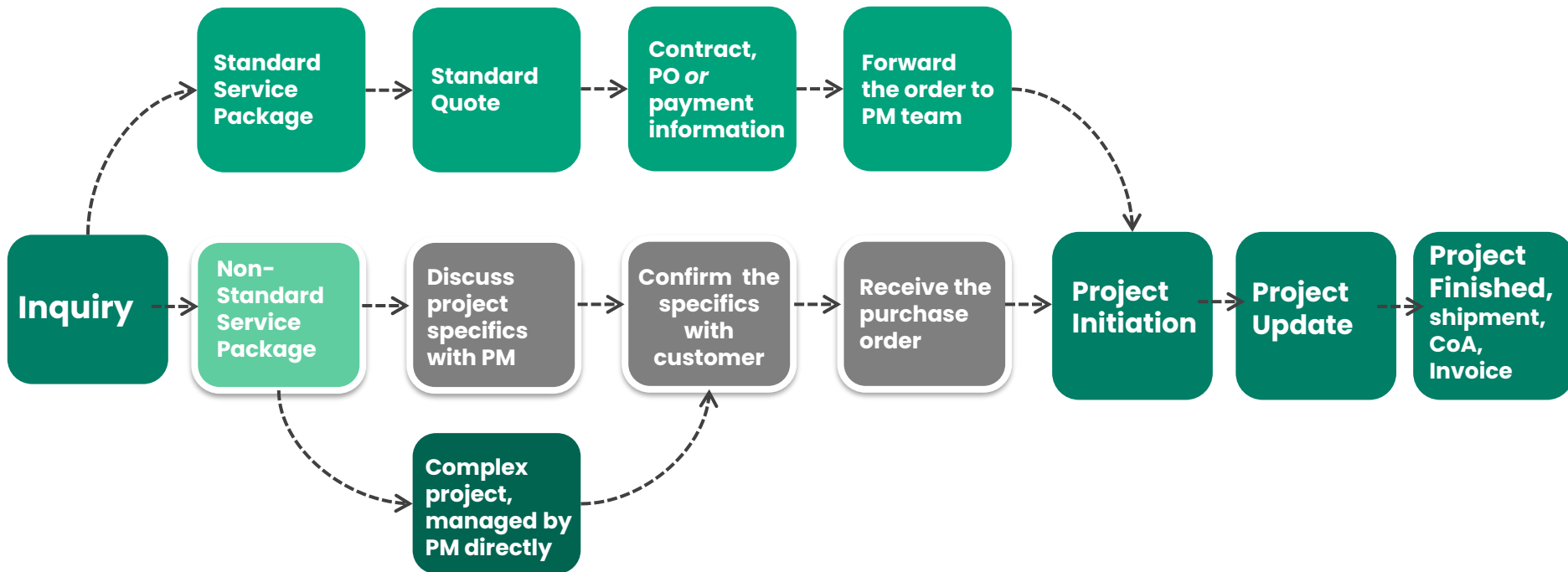


Single B-cell Based  
Platform



Rabbit pAb Development

## CRO Service General Workflow

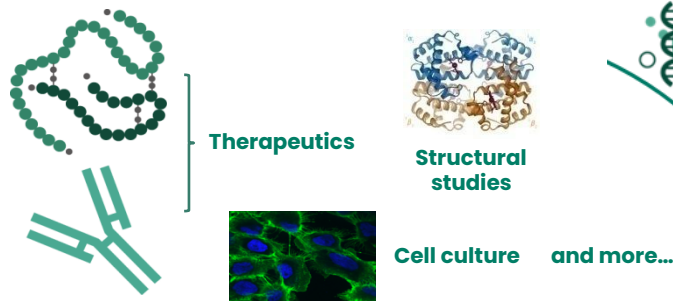


# 01

## Recombinant Protein Expression

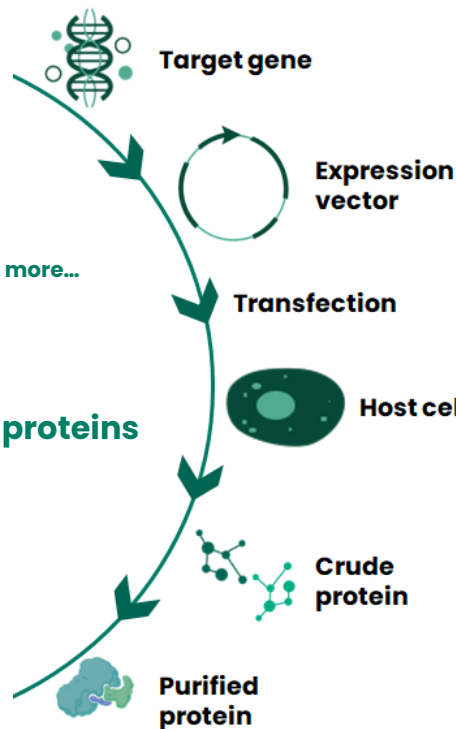
# Concept and Host Systems

## Recombinant proteins for...

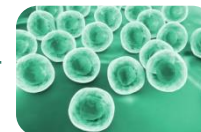


## Natural proteins vs. Recombinant proteins

	Natural	Recombinant
Source	Limited	Unlimited
Contamination	Yes	Animal-free
Yield	Low	High
Batch variations	High	Controllable

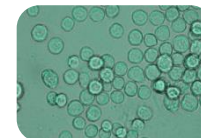


\*PTM = Post translational modification



HEK293/CHO

- Correct PTM\*, soluble proteins
- Long culture duration, costly



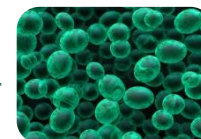
Insect cell

- PTM\*, high cell density, soluble proteins
- Partial glycosylation, costly



*E. coli*

- Low cost, rapid expression, easy to scale-up
- No PTM\*, inclusion bodies, MW limitation

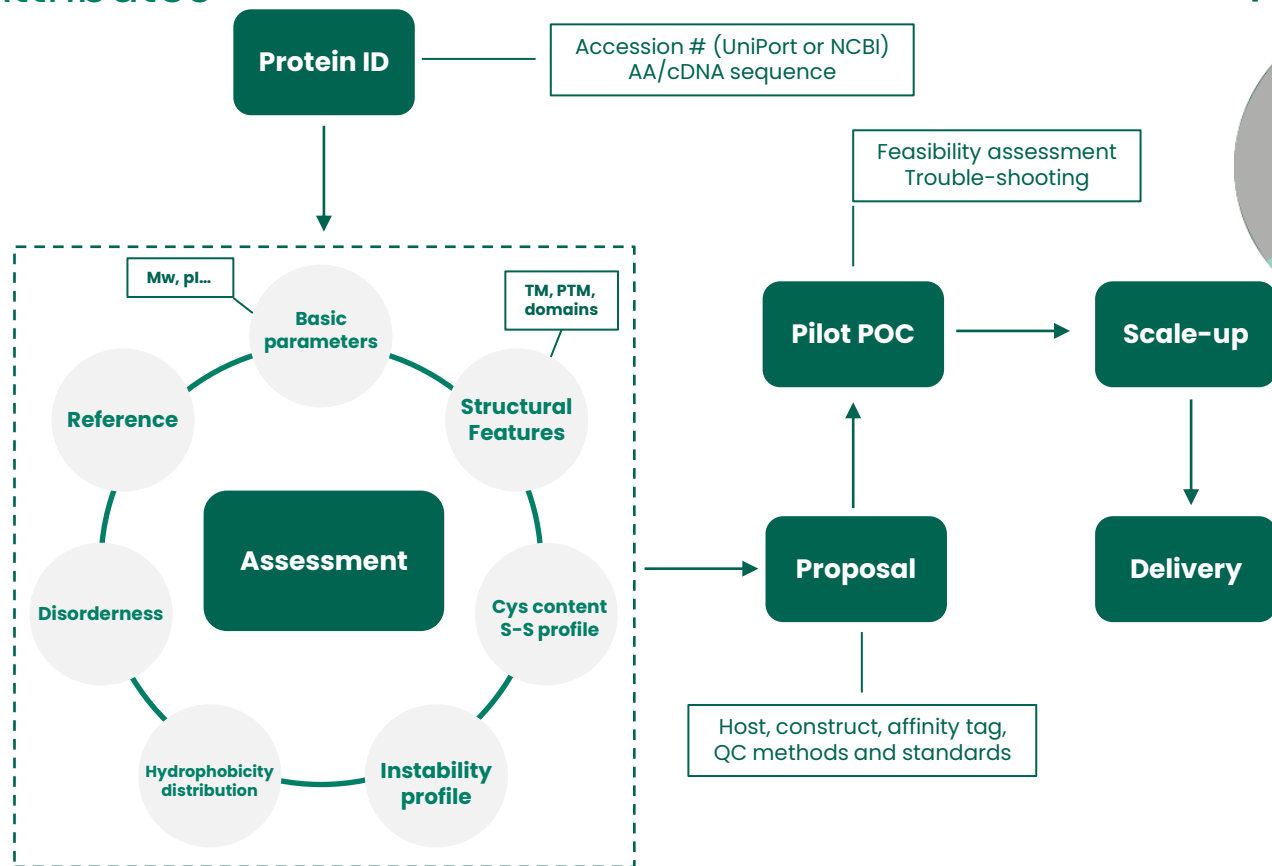


Yeast

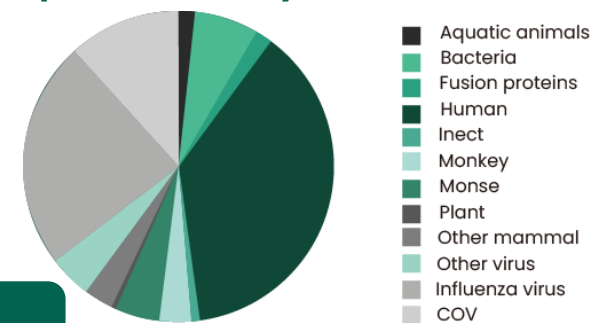
- Low cost, rapid expression, easy to scale-up
- PTM\* with high mannose content glycans



## Attributes



## Species diversity



Host species of protein-of-interest, 2019-2020



**Affinity Tags (\*\* major purification tags, \*\* purification feasible, \* purification feasible but not recommended)**

Tag	Size	Suitable Hosts	Application Notes
His***	(His)6~10	Universal	<ul style="list-style-type: none"><li>• Small MW, low immunogenicity, low impact on protein function</li><li>• Limited solubility enhancement</li><li>• Suitable for denature purification methods</li></ul>
FLAG*	DYKDDDDK	Insect and mammalian	<ul style="list-style-type: none"><li>• Small MW, low immunogenicity, low impact on protein function</li><li>• Limited solubility enhancement</li><li>• DDDDK= EK digestion site to obtain Nt tag-free protein</li><li>• Detection tag, commonly used in a His-FLAG dual tag fashion</li></ul>
GST**	~28 KDa	Insect and <i>E.coli</i>	<ul style="list-style-type: none"><li>• Enhance solubility with certain impact on protein function (spatial hindrance)</li><li>• Purification tag</li><li>• Not suitable for denature purification methods</li><li>• Limited stability: prone to degradation</li></ul>
MBP	~42 KDa	Insect and <i>E.coli</i>	<ul style="list-style-type: none"><li>• Enhance solubility with improved stability. Impact protein function</li><li>• Not a purification tag, commonly used in a His-MBP dual tag fashion</li><li>• Not suitable for denature purification methods</li></ul>
Fc***	~25 KDa (dimmer)	Insect and mammalian	<ul style="list-style-type: none"><li>• Enhance solubility and extend protein serum half-life</li><li>• Purification tag, low pH elution method</li><li>• Not suitable for denature purification methods</li><li>• Limited stability: prone to aggregation</li></ul>
SUMO and Trx	~12 KDa	<i>E.coli</i>	<ul style="list-style-type: none"><li>• Enhance solubility with certain impact protein function</li><li>• Not a purification tag, commonly used in a His-SUMO/Trx dual tag fashion</li><li>• Tag removal is usually required</li><li>• Not suitable for denature purification methods</li></ul>

# Case Study: Homo-dimer Expression

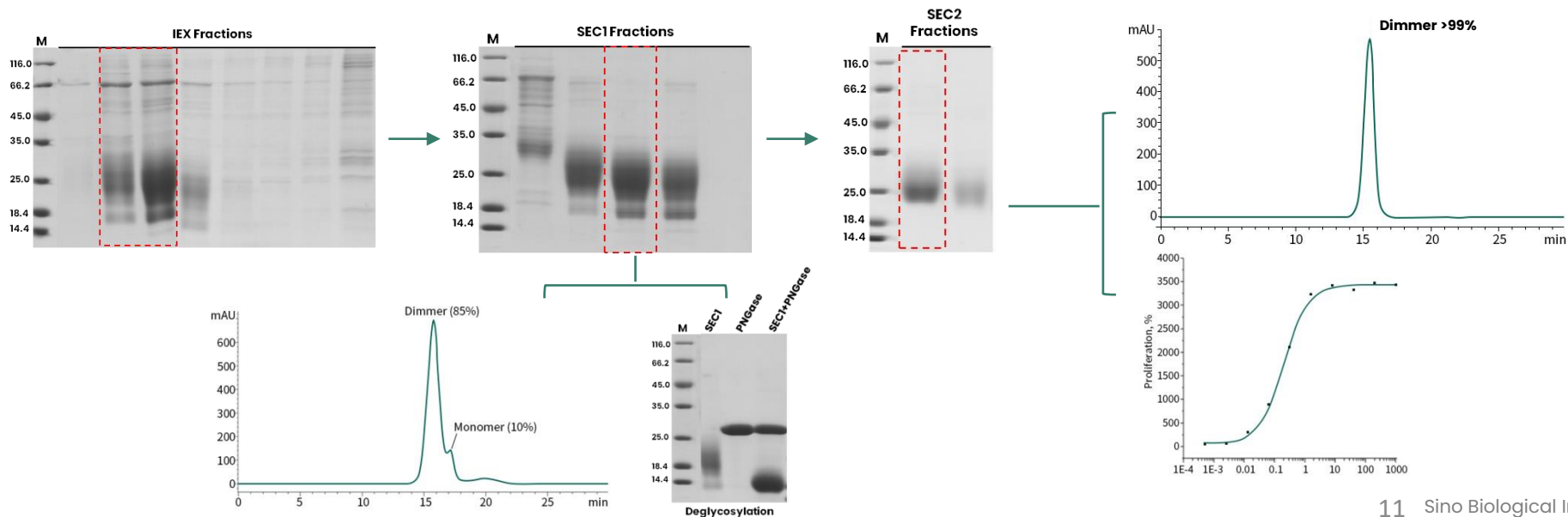
Aim: tag-free homo-dimer protein (monomer MW= $\sim$ 20KDa)

Host: HEK293

Attributes: active protein with mg quantity, >95% purity (SEC+PAGE), homo-dimer

## Purification Process Development

Ion exchange (IEX)  $\longrightarrow$  Gel-filtration 1 (SEC 1)  $\longrightarrow$  Quality assessment  $\longrightarrow$  Gel-filtration 2 (SEC 2)  $\longrightarrow$  Quality assessment



# Case Study: ECD of a secreted protein

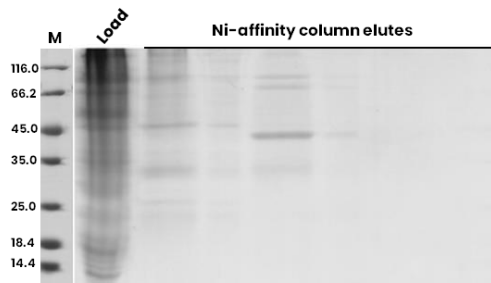
Aim: produce ECD of a single-pass membrane protein (ECD MW $\approx$ 90KDa)

Host: Insect cells

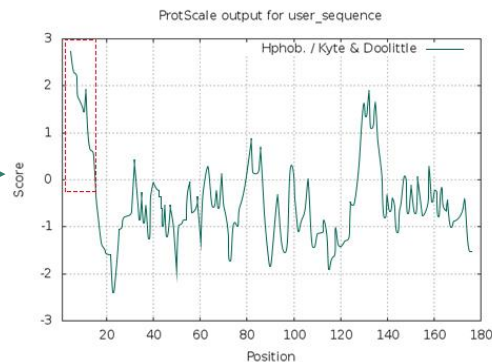
Attributes: >95% purity, stable, secreted protein

## Construct Optimization

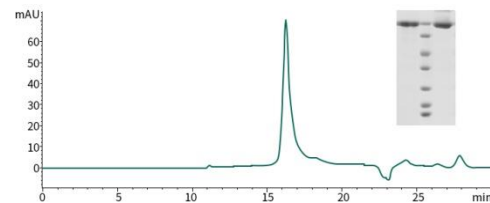
Construct 1: full ECD with Nt His tag



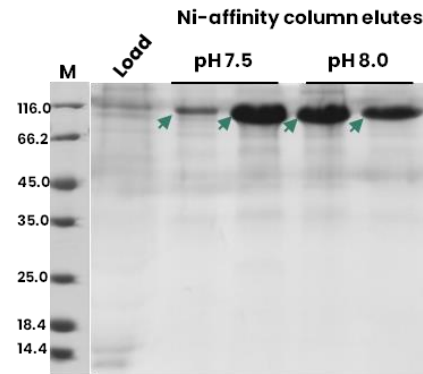
Hydrophobicity analysis



Revise construct



Construct 2: remove Nt hydrophobic region



# Case Study: A single-pass trans-membrane protein

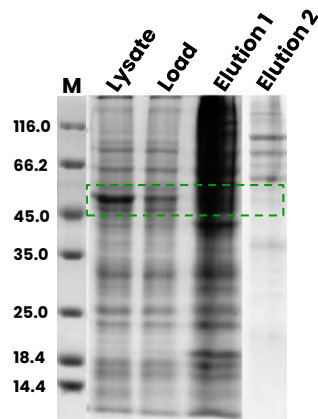
Aim: membrane protein production (Protein MW= $\sim$ 61 KDa)

Host: Insect cells

Attributes: >90% purity, TM, detergent, hydrophobic protein

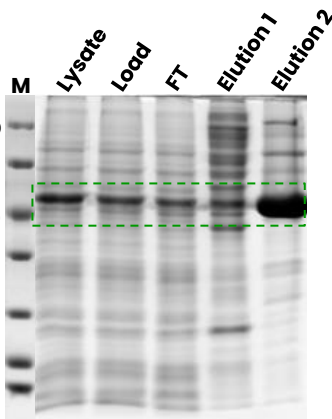
## Buffer Optimization

Buffer content: PBS  
Detergent: Triton X-100



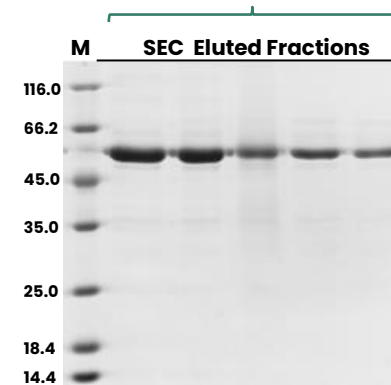
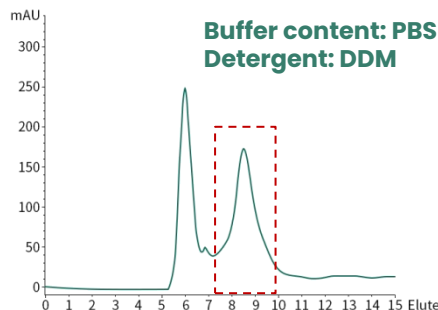
Ni-affinity purification

Buffer content: PBS  
Detergent: Triton X-100  
and tween 20



Ni-affinity purification

Polishing



SEC purification

# Case Study: SARS-CoV-2 Nucleocapsid Protein

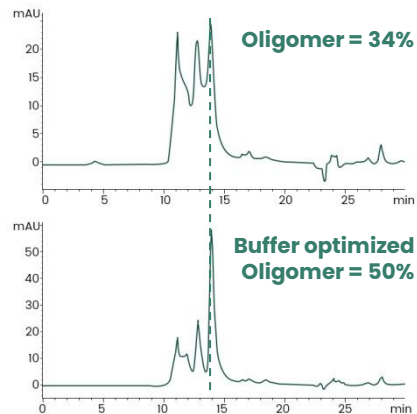
Aim: produce SARS-CoV-2 NP (MW of monomer= $\sim$ 45kDa)

Host: Insect cells, *E.coli*

Attributes: >95% purity, stable, oligomeric protein

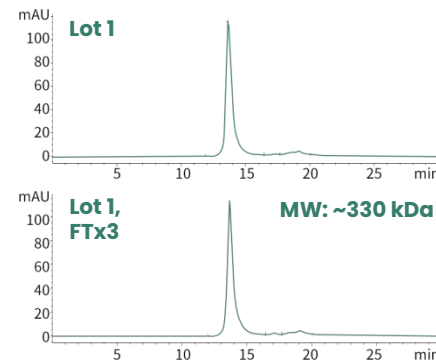
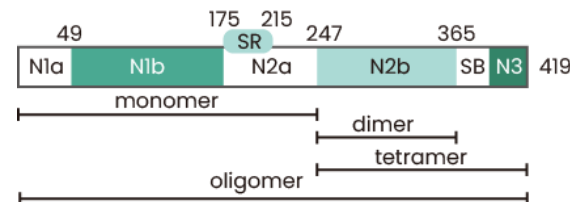
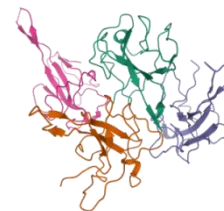
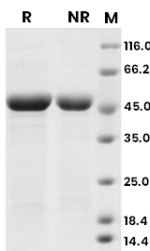
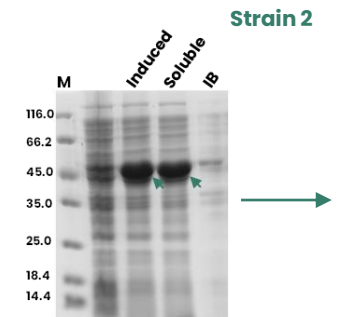
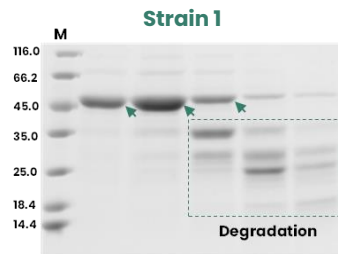
## Host and Construct Optimizations

Host: Insect cells



Switch host  
*E.coli*

Host: *E.coli*

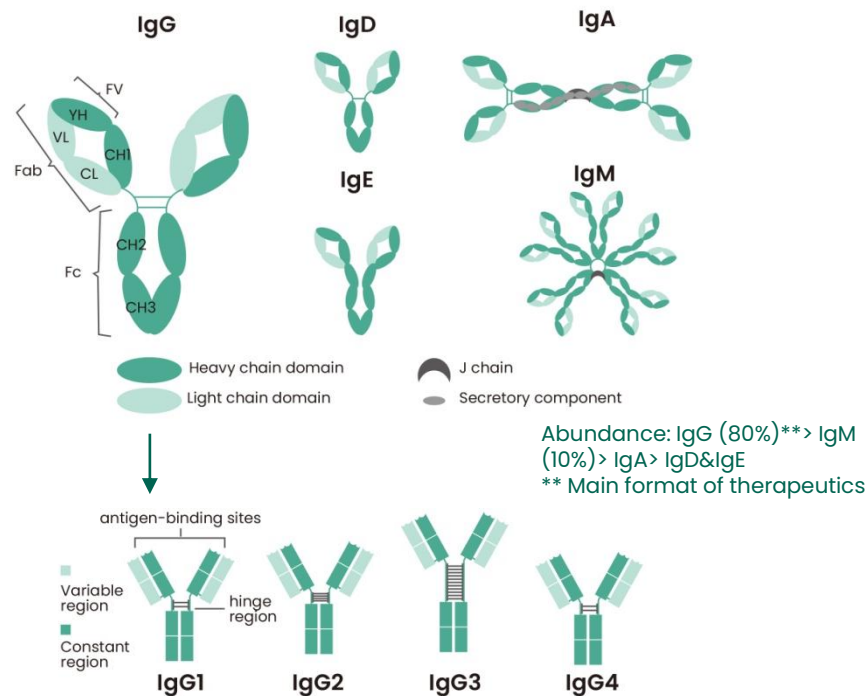


# 02

## Recombinant Antibody Expression

# Recombinant Antibody, Shapes and Formats

## Full-length Ab



## Fragmented Ab



## Ab fusion proteins



## Chimeric Ab



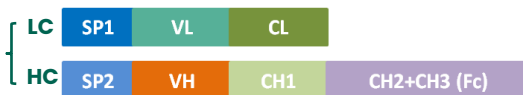
## Bi-specific Ab





# Recombinant Antibody Expression: Methods

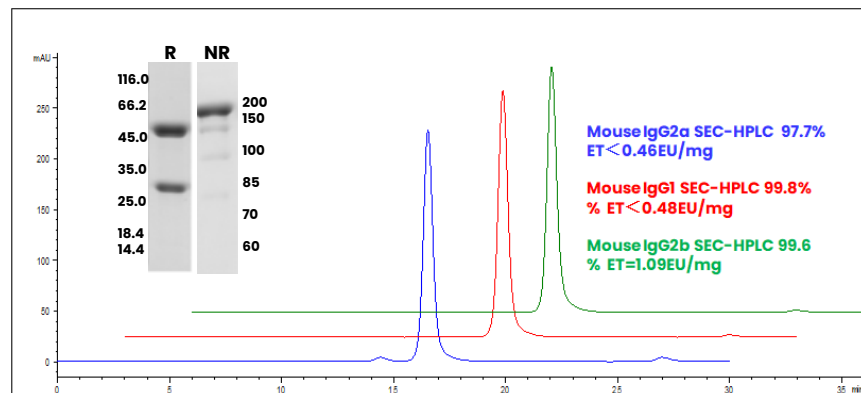
- Expression host: HEK293 or CHO
- Culture method: co-transfection
- Purification tag: Fc or His (scFv, VHH, Fab)



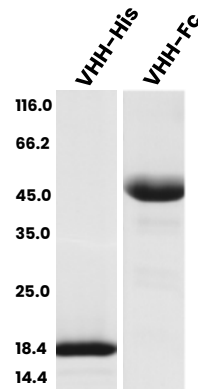
## Bi-specific Ab

Format	# of Ab
BiTE	>5
CrossMab	3
Diabody-Fc	2
DutaMab	>15
DVD/DVI-IgG	>5
IgG(H/L)-scFv	>5
VHH1-Linker-VHH2-Fc	3

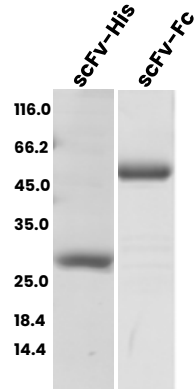
## Recombinant full-length Ab



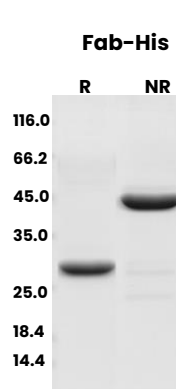
## Recombinant VHH



## Recombinant scFv



## Recombinant Fab

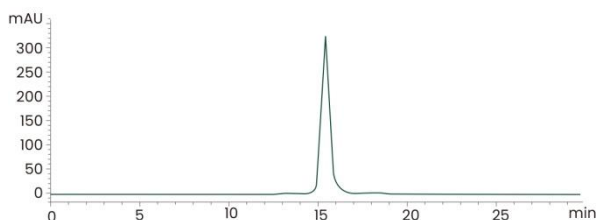
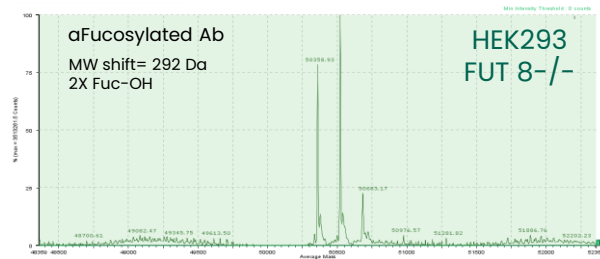
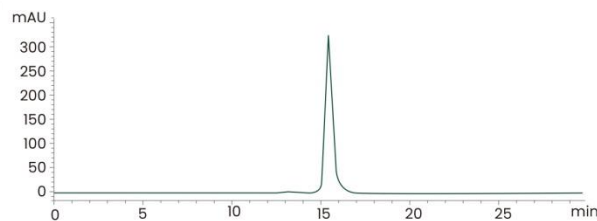
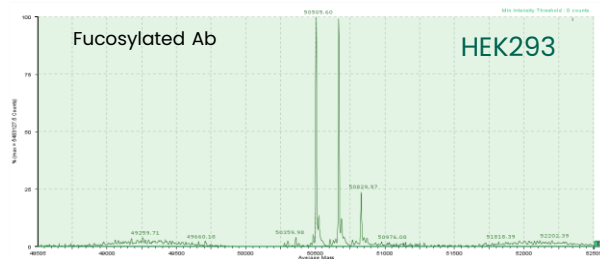


# Featured Service, $\alpha$ Fucosylated Ab

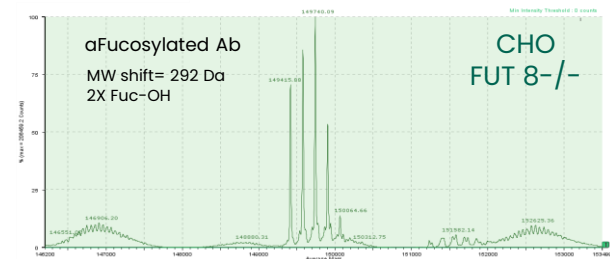
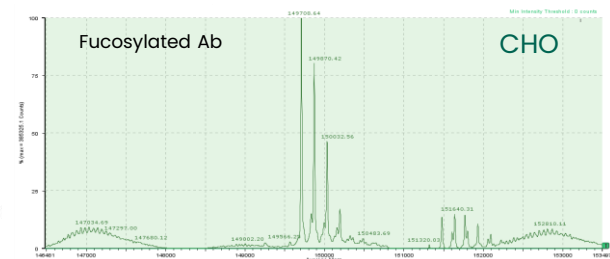
**FUT8  $-/-$  HEK293 and CHO cell lines**

Afucosylated Ab for ADCC enhancement

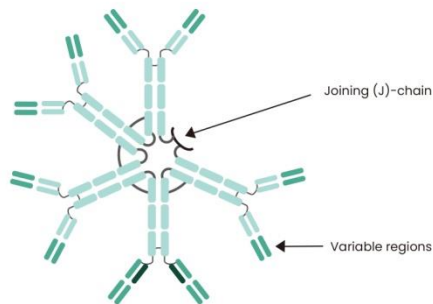
Full-length antibodies expressed by HEK293 cells



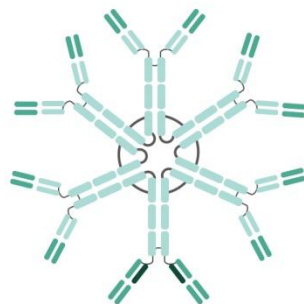
Full-length antibodies expressed by CHO cells



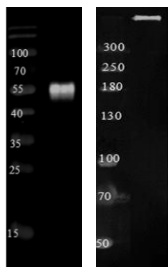
# Featured Service, IgM Production



W/ J chain-pentamer



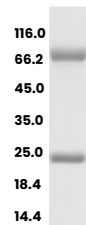
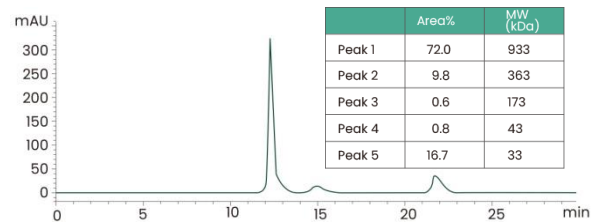
W/out J chain-hexamer



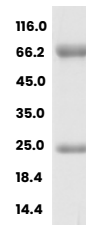
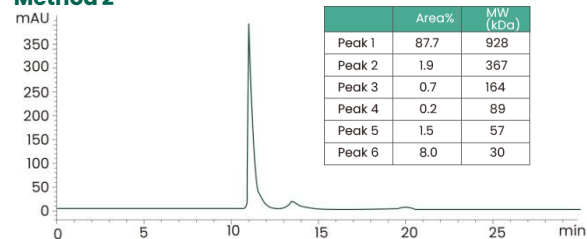
J chain maybe associated with heavy chain in a S-S independent manner

## Pentameric IgM

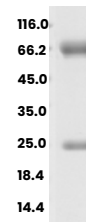
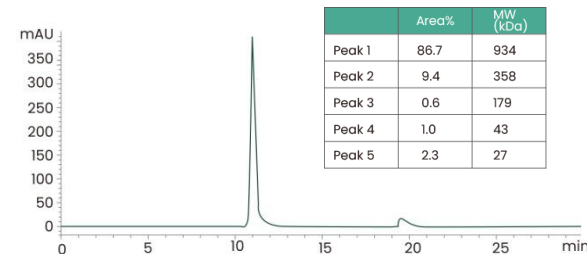
### Method 1



### Method 2



## Hexameric IgM



# Lead time for recombinant protein/Ab expression projects

## ***E.coli*: protein expression**

Gene synthesis	Vector construction	Feasibility Assessment	Scale-up	QC and delivery
1~4 weeks	1~2 week	1~2 week	1~2 weeks	~1 week

## ***Insect*: protein expression**

Gene synthesis	Vector construction	Virus packaging	Feasibility Assessment	Scale-up	QC and delivery
1~4 weeks	1~2 week	1~2 week	1~2 week	1~2 weeks	~1 week

## ***Mammalian*: protein and antibody expression**

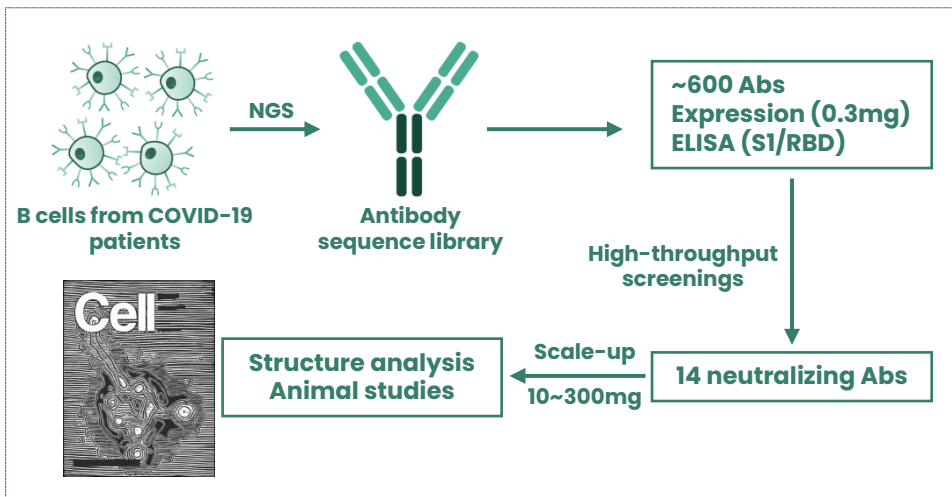
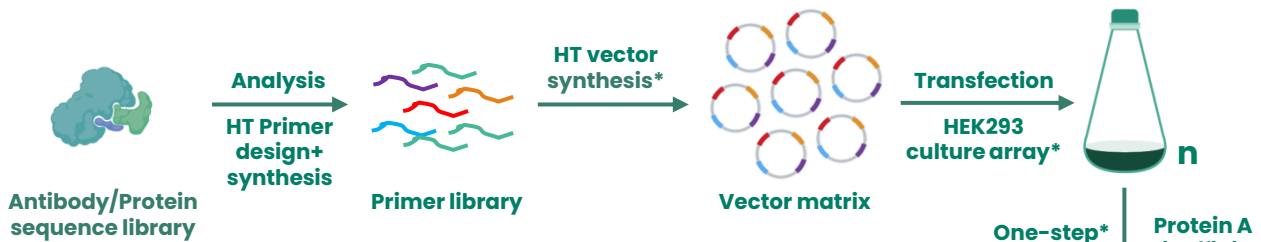
Gene synthesis	Vector construction	Feasibility Assessment	Scale-up	QC and delivery
1~4 weeks	1~2 week	1~2 week	1~2 weeks	~1 week

### **Note:**

The lead time of gene synthesis depends on the size of the target gene

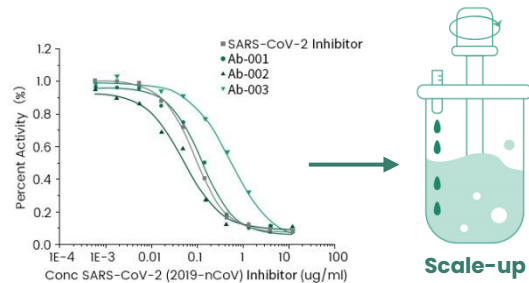
- <2000bp-- 1-2 weeks
- >2000bp-- 2-4 weeks

# High-throughput Expression Platform



## Status

- **Volume:** 20~400 mL (flasks)
- **Weekly culture capacity:** 100~200
- **Ave Ab success rate:** >99%
- **Fast Turnaround:** 2~4 weeks from gene synthesis to purified antibody
- **Virus proteins:** HA, NA, RBD MT



## Lead time for high-throughput Ab Expression Service

Quantity	Timeline	Description	Deliverables
100 ug	3 weeks+	<ul style="list-style-type: none"><li>• Gene synthesis</li><li>• Vector construction</li><li>• Sequencing confirmation</li><li>• Plasmid preparation</li><li>• Transient transfection of HEK293/CHO cells</li><li>• Purification and QC analysis</li><li>• additional analysis (optional)</li></ul>	<ul style="list-style-type: none"><li>• Purified antibody</li><li>• COA</li></ul>
500 ug			
1 mg			

- ✓ Full length human IgG1, human IgG4, mouse IgG2a, mouse IgG2b
- ✓ Minimum order quantities: 5 samples
- ✓ Standard analysis: concentration (UV); purity (SDS-PAGE).
- ✓ Optional analysis: SEC-HPLC, Endotoxin, Mass Spec, Elisa, FACS, Affinity by BLI/SPR, etc.

# 03

## Antibody Development

# Comprehensive Antibody Technologies

## Antibody Engineering

- Antibody Sequencing
- Antibody Humanization

## Screening and Evaluation

- Immunoassays: ELISA, FACS, WB, IHC, IF, IP
- Biochemical & Biophysical Characterization: BLI, SPR, SEC
- *In Vitro* & *In Vivo* Bioactivity Assays

## Optimized Immunization

- Protein Immunization
- Peptide/Fragment Immunization
- DNA Immunization
- Whole Cell Immunization

## Development & Production

- Classical Mouse mAb Technology
- Phage Display Rabbit mAb Technology
- Single B Cell-based Antibody Technology
- Rabbit pAb Development
- Anti-idiotypic Antibodies
- Phospho-specific Antibodies
- Proprietary Mammalian Platform Supports High-throughput & Large-scale Recombinant Antibody Production

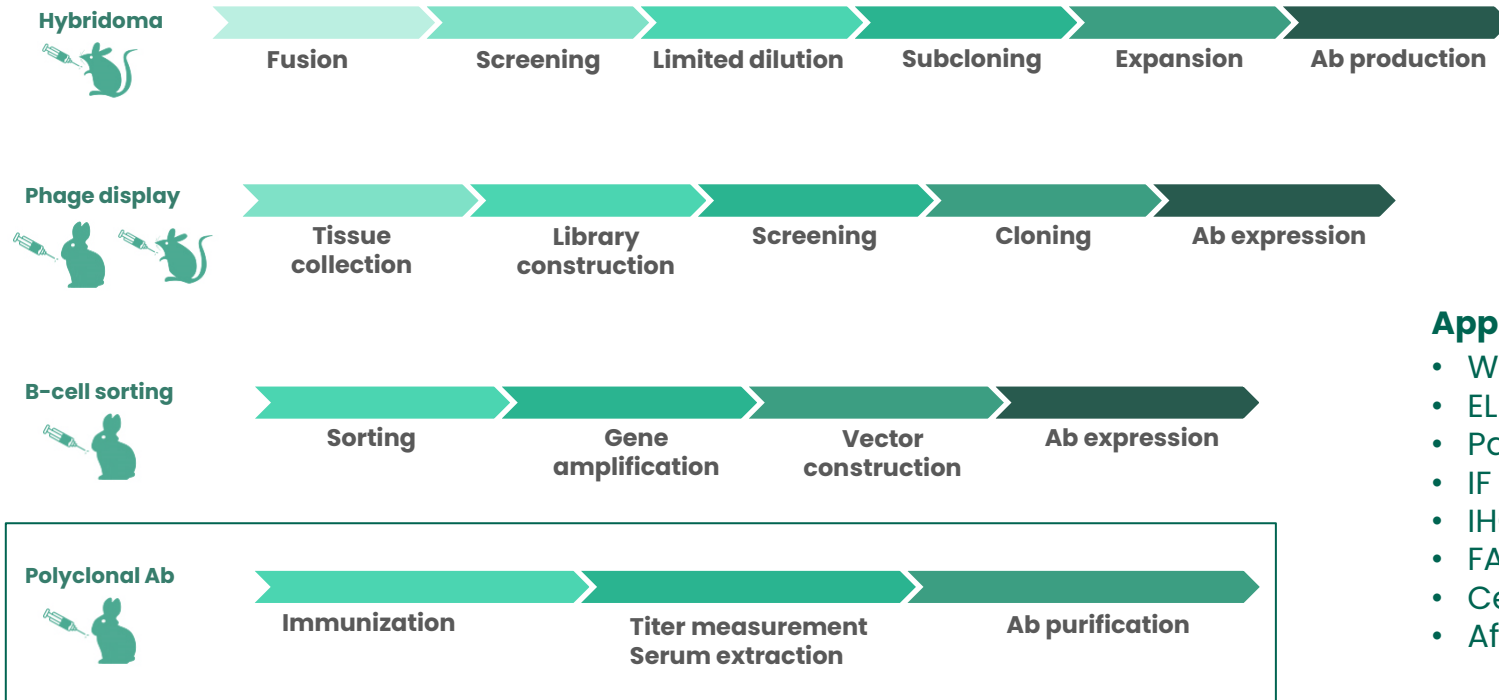


**Antibody  
Technologies**



# Platforms for antibody development

## Methods for monoclonal Ab discovery (after immunization)



## Antigen formats:

- Peptide
- Recombinant protein
- DNA
- Whole Cell

## Applications for Ab:

- WB
- ELISA
- Paired ELISA
- IF
- IHC
- FACS
- Cellular functions
- Affinity purification

## General lead time

Service Package	Workflow	Time line
Standard Polyclonal Antibody Services	2 rabbits immunization-Protein A Purified/Affinity Purified	10-14 weeks
	Peptide synthesis-2 rabbits immunization-Protein A Purified/Affinity Purified	14-20 weeks
	Antigen expression-2 rabbits immunization-Protein A Purified/Affinity Purified	14-20 weeks
Speedy Polyclonal Antibody Services	2 rabbits immunization-Protein A Purified/Affinity Purified	45 days
Phospho-Specific Polyclonal Antibody Services	Peptide synthesis-2 rabbits immunization-Protein A Purified/Affinity Purified	14-20 weeks
Mouse Monoclonal Antibody Services	5 mice immunization-fusion and screening-antibody production & purification	Standard 4-6 months Speedy 3-4 months
Rabbit Monoclonal Antibody Services	2 rabbits immunization-Phage display library-screening-antibody production & purification	Standard 4-6 months Speedy 3-4 months

# Case Study – TIGIT Antibody Discovery

## Workflow

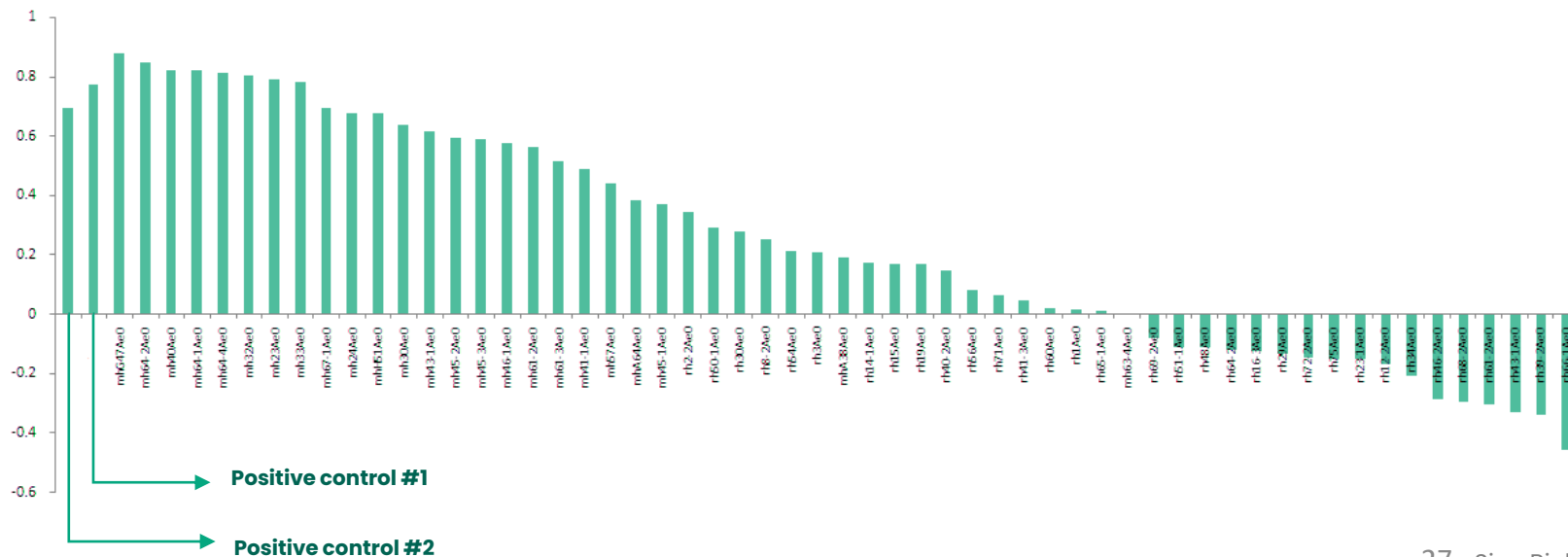
Antigen specific B cell sorting

Single cell PCR

Antibody Expression

Validation

Antigen-Specific B cells	VH/VL Paired Sequences	ELISA Positive Clones	Positive Rate	Competition Assay	Workflow ( after immunization)
380	142	46	32.4%	8 (better than control)	26 days



# 04

## CRO for Virology Research

# ProVir™ Virus Research Reagents

50+ Viruses	AcMNPV	CHIKV	CHPV	Coxsackievirus	Cymv
	Cytomegalovirus	DcCoV	Dengue virus	Ebola virus	EBV
	EV71	EV-D68	GTOV	HAdV-B	HAdV-E
	Hepatitis B virus	HCoV-229E	HCoV-HKU1	HCoV-NL63	HCoV-OC43
	HCV	HEV	HIV	HPV	HPIV-3
350+ Strains	HPIV-4	HTNV	Influenza virus	JUNV	LASV
	LCMV	LUJV	MARV	MERS-CoV	NIV
	PCV2	PRRSV	RSV	RVFV	SABV
	SARS-CoV	SARS-CoV-2	SFTSV	SIV	TBEV
	Vaccinia virus	VSIV	WNV	WWAV	Zika virus
800+ Antigens					
3000+ Reagents					

<https://www.sinobiological.com/research/virus>

## • Fast response

2013-4: H7N9 HA & NA – 12 days

2016-6: Zika virus proteins – 14 days

2020-1: SARS-CoV-2 RBD – 12 days

**2021-12: SARS-CoV-2 RBD (Omicron) – 6 days**

## • Comprehensive collections

Upper respiratory virus antigen array

Influenza vaccine strain HA (2015-2021+)

SARS-CoV-2 Spike MT (>20 SI MT, >140 RBD MT) **B.1 (D614G)**

**B.1.1.7**

**B.1.351**

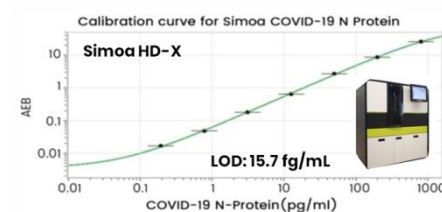
**B.1.429**

**P.1**

**Mink**

**B.1.617**

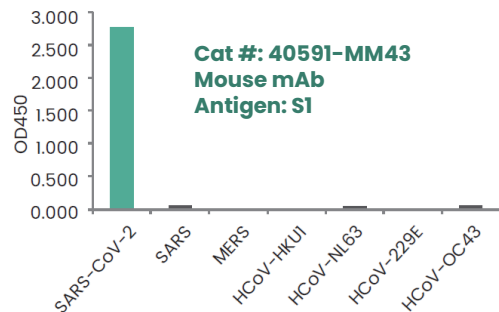
**B.1.1.529**



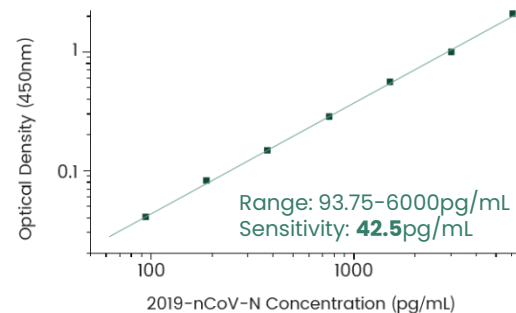
## • Hyper sensitive

# Virus-specific Antibody Development

## High specificity

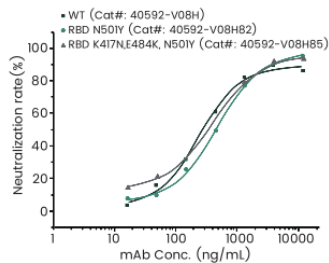


## High sensitivity

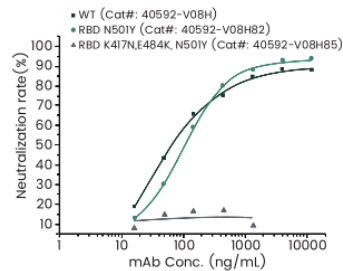


## SARS-COV 2 neutralizing Ab

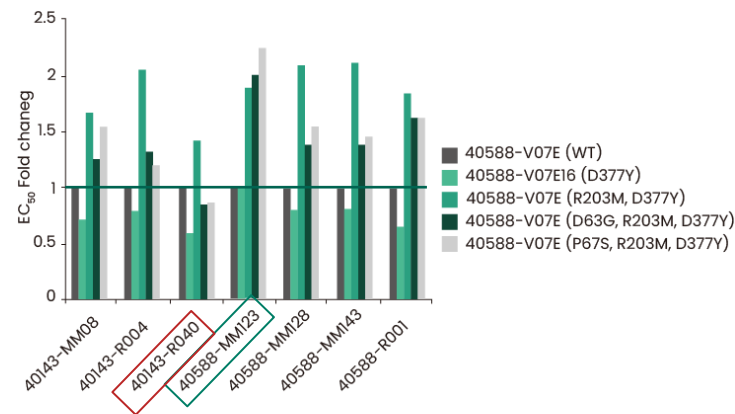
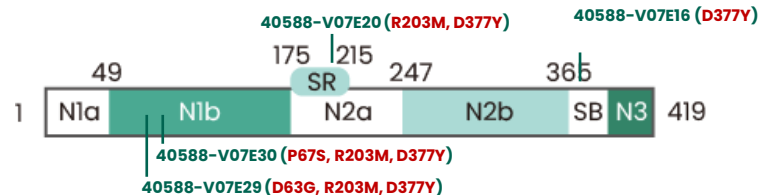
### Spike Neutralizing Antibody (Cat#: 40150-D001)



### Spike Neutralizing Antibody (Cat#: 40592-R118)



## Broad spectrum: Anti-NP mAb against B.1.617 Variant Proteins



# THANK YOU



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