Sanyou Bio
Biological Drug R&D
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Mouse/Alpaca immune antibody library screening service

Accelerating the Development of Innovative Antibody Drugs for Challenging Targets

Innovation Outstanding Reliability

Sanyou Biopharmaceuticals Co., Ltd.

CONTENTS

Service Overview

——Mouse/Alpaca Immunization Ab Library

——Service Highlights

——Service Process

Mouse/Alpaca Immunization Ab Library

Sanyou Bio. is the earliest company to provide screening services for mouse Immunization Ab Library in China

- It can be widely used in the fields of innovative drug discovery, diagnosis, detection, and antibody customization for scientific research
- It can cover the needs of molecular discovery such as "**single domain antibody, mouse monoclonal antibody**, bispecific antibody, polyclonal antibody"
- Can quickly and successfully obtain candidate molecules with high affinity, excellent specificity, and good developability

Mouse/Alpaca immune antibody library

- ✓ Early sequence acquiring that save 2-3 months compared with hybridoma technology
- ✓ Diversified antigens, mouse strain, immune methods and cross-screening methods
- ✓ Median lead antibody number : 40+

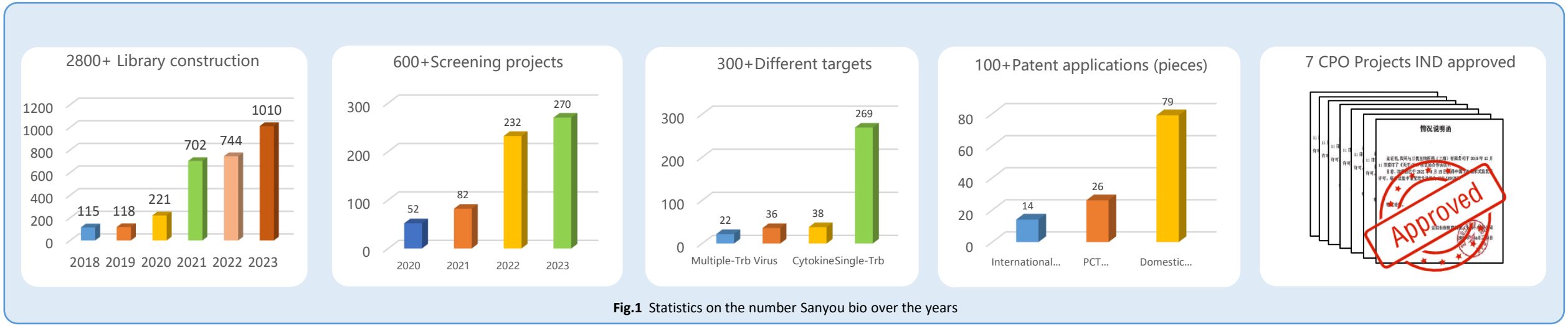


Fig.1 Statistics on the number Sanyou bio over the years

Service Highlights

Short screening cycle

Only 4-5 weeks to construct and cross-screen Immunization Ab libraries to obtain unique sequences after diversity analysis.

Service Phase	Service content	Cycle
1. Antigen preparation (optional)	◆ Antigen protein expression (eukaryotic/prokaryotic/insect), polypeptide/DNA preparation	1-2 w
2. Mice immunization (optional)	◆ Multi-path immunity customization ◆ Mouse serum titer detection	7-9 w
3. Immunized library construction and screening	◆ **Phage display library construction** ◆ **Panning and screening** ◆ **Sequencing and diversity analysis**	**4-5 w**
4. Eukaryotic verification	◆ CHO/HEK293 eukaryotic expression ◆ ELISA, FACS, SDS-PAGE, SEC, and other preliminary drug developability analysis	1-2 w
5. Molecular activity verification (optional)	◆ ELISA / FACS species cross-binding assay ◆ ELISA/FACS blocking activity assay ◆ Affinity Kinetics (BLI/SPR) (BLI / SPR)	1-2 w

High number of lead antibodies

A large number of antibody clones can be obtained from the mouse immunized antibody library . This library was validated by screening of 12 targets, and a total of 637 antibody clones with unique sequences were obtained with a median number of 55 clones.

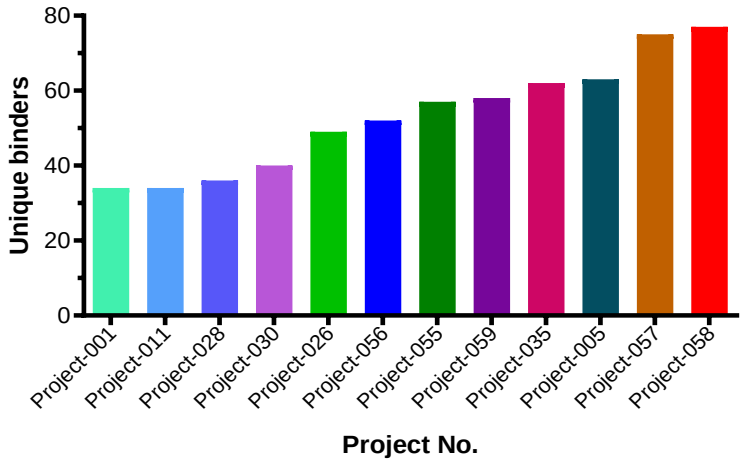


Fig. 2 Antibody number per project

Service Highlights

High affinity of lead antibodies

The affinity of antibodies obtained from the mouse antibody library could usually reach to the pM level. The antibodies in Fig. 3 and Fig. 4 are from two different projects, and both projects yielded dozens of antibodies with affinity comparable to or significantly superior to that of the reference antibody (benchmark).

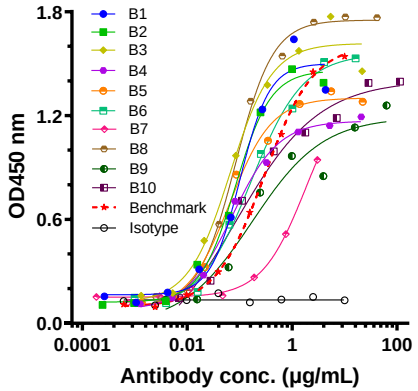


Fig. 3 Affinity Ranking of MIT-051

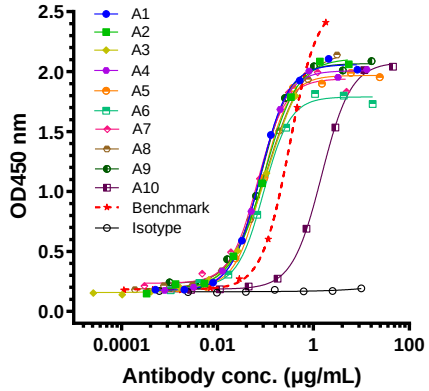


Fig. 4 Affinity ranking of MIT-056

Comprehensive drug ability analysis

After full-length construction of the molecules obtained through the mouse immunized antibody library screening, the expression level and physiochemical characteristics of the antibody were comprehensively analyzed. As shown in Table, the analysis covers multiple aspects such as purity and concentration determination, primary structure analysis, affinity, and affinity kinetics.

Category	Test	Test method
Purity and concentration detection	Purity identification	SDS-PAGE/SEC/CE-SDS
	Concentration identification	Protein A-HPLC/UV280
Primary structure analysis	Molecular weight analysis	LC/MS
	Isoelectric point	iCIEF
	Hydrophobicity identification	HIC-HPLC
	Charge heterogeneity determination	CEX
	Peptide mapping analysis	LC-UV-MS/MS
	N-glycan mapping analysis	LC/MS
Affinity and affinity kinetics	Affinity	ELISA
	Affinity kinetics	BLI/SPR
	Cellular binding assay (demand-based)	FACS

Service Process

Material QC	**Antigen screening**: SDS-PAGE purity identification, ELISA activity identification, and Biotin labeling **Cell screening**: mycoplasma test and FACS target antigen expression identification **Reference antibody (benchmark)**: SDS-PAGE purity identification, SEC-HPLC monomer purity identification, and ELISA activity identification	1 week
Immunization	**Immunization strategies**: protein immunization, cellular immunization, and protein-celluar cross immunization **Immunization regimens**: His immunization, Fc immunization, His antigen & Fc antigen cross immunization, and protein-cellular cross immunization	7-9 weeks
Library construction	**Library construction strategy**: array-based library construction **Quality control**: sequencing, library capacity>10^8	4-5 weeks
3-round panning	**Panning library**: mouse immune library **Panning strategies**: protein panning, cell panning **Panning regimens**: solid phase, liquid phase, cells, solid-liquid cross-panning, His antigen & Fc antigen cross panning, and protein-cell cross panning	
screening	**Screening method**: Fab/phage ELISA, Fab/phage FACS **Screening scale**: 20+ pool, 800-1000 clones for initial screening	
Sequencing analysis	200+ clones for sequencing and analyze unique sequence diversity via AI program	
Mammalian cell validation	30-60 antibody molecules, mammalian cell expression, and physicochemical identification	2-3 weeks

Representative Cases

——Monoclonal antibody development of GPCR target

——ADC drug development of target A

Monoclonal antibody development of GPCR target

Target information

Difficulties in GPCRs antibody drug development:

1. Low expression and high cost of material preparation.
2. Complex structure and highly hydrophobic, high difficulty in purification.
3. Fewer extracellular domain, and more difficult to screen for functional antibodies.

Therefore, the current drug development is mainly focused on small molecule drugs or low molecular weight peptides.

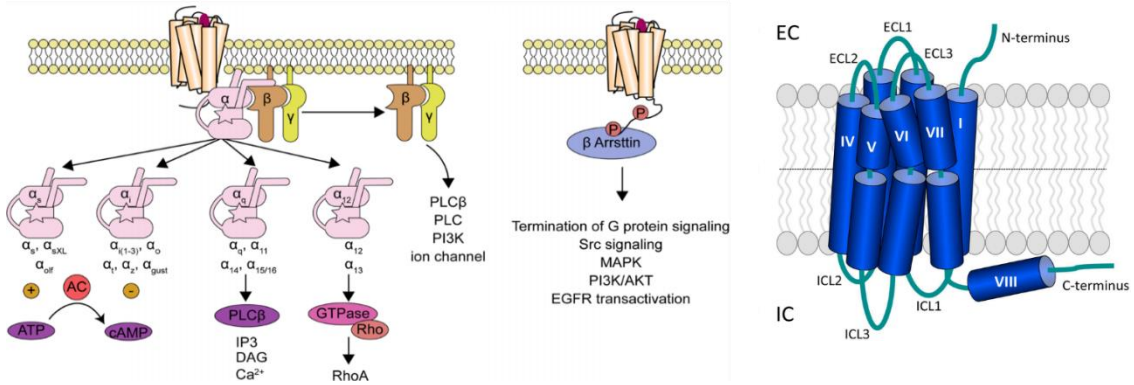


Fig. 5 Mechanisms of GPCRs

[1] Recent progress in assays for GPCR drug discovery. Am J Physiol Cell Physiol. 2022;323(2):C583-C594

[2] Modulation of cellular signaling by herpesvirus-encoded G protein-coupled receptors. Front Pharmacol. 2015;6:40.

Technology Route

1	2	3	4
Material Identification	Mouse Immunization	Panning and Screening	Screening
Protein：VLP Cells：overexpression cells、tumor cell line Benchmark（BM）	Protein immunization Cell immunization Protein-cellular cross immunization	Solid phase, cells panning Fab/phage ELISA, Fab/phage FACS Sequencing	FACS Affinity kinetic assay

Monoclonal antibody development of GPCR target

Analysis of binding affinity

As shown in Fig. 6, the affinity assay of candidate antibodies to tumor cells was analyzed by FACS, and the results showed that most of the candidate antibodies obtained by mouse immunized library had better affinity than benchmarks.

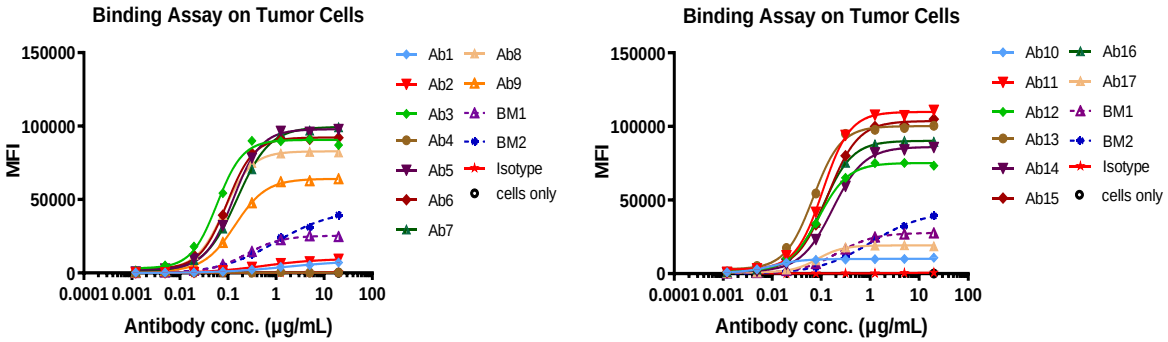


Fig. 6 Binding affinity determination by FACS

In vitro pharmacodynamics

As shown in Fig. 7, the blocking ability of candidate antibodies on tumor cell migration was analyzed by FACS, and most of the candidate antibodies obtained by mouse immunized library had good blocking activity.

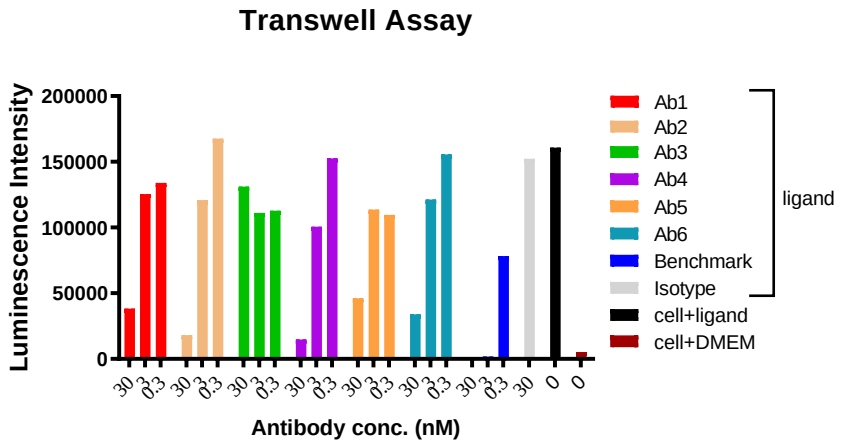


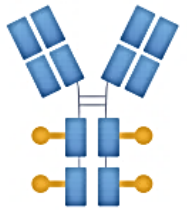
Fig. 7 Transwell determination by FACS

ADC drug development of target A

Target information

Background: Target A is a single transmembrane protein that is highly expressed in a variety of tumor types, including triple-negative breast and pancreatic cancers, and its high expression is associated with pool survival These characteristics make target A an ideal target for tumor-targeted therapeutic drug development.

MOA: Anti-target A monoclonal antibodies bind to antigens on the surface of tumor cells to induce internalization of target A, which delivers cytotoxic drugs into the cell and kills tumor cells. In addition, monoclonal antibodies targeting monoclonal antibodies against target A can also direct immune effector cells to tumor cells and kill them.



ADC

Technology Route

1	2	3	4	5	4
Material Identification	Mouse Immunization	Panning and Screening	Screening	*In vitro* assay	*In vivo* assay
Protein：A-His / huFc protein Cell：A-HEK293 / CHO-S Benchmark（BM）	Protein immunization Cell immunization Cross immunization	solid phase, liquid phase, cells, protein-cell cross panning Fab/phage ELISA Sequencing	ELISA, FACS Affinity kinetic assay	Endocytosis assay Cell killing assay	Endocytosis assay ADC killing assay

ADC drug development of target A

Analysis of binding affinity

Fig. 8 demonstrates the results of the affinity assay of the candidate antibodies with tumor cells by FACS, and the candidate antibodies exhibit high affinity.

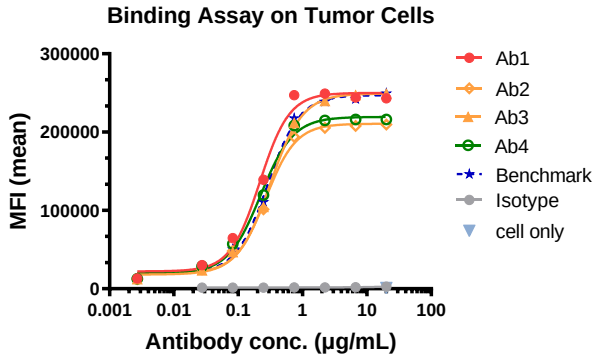


Fig. 8 Binding affinity determination by FACS

ADC Killing assay

Fig. 9 shows the toxicity analysis after candidate antibody-mediated endocytosis of the toxin delivered into the cell. The results show that the candidate antibody mediates the killing of antigen overexpressing cells by the toxin with an effect comparable to that of the benchmark.

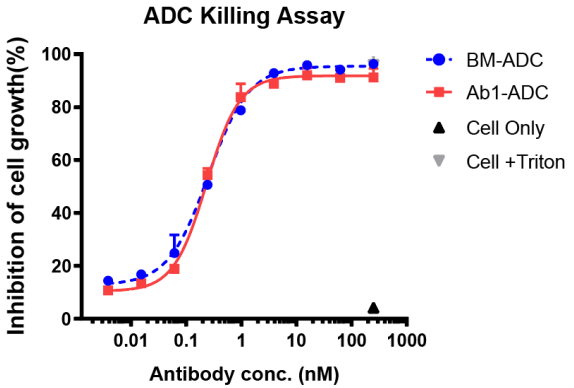


Fig. 9 Cytotoxicity determination by FACS

In vivo pharmacodynamics

As shown in Fig. 10, the N87 subcutaneous tumor model was constructed using BALB/c nude mice. After multiple doses, Ab1 ADC exhibited similar tumor suppressive activity as the benchmark ADC drug.

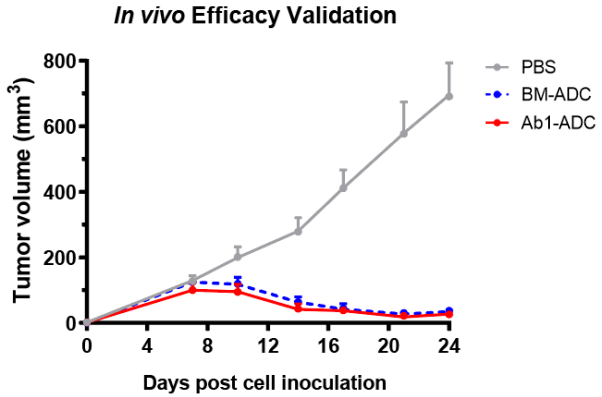


Fig. 10 Tumor growth inhibition

Deliverables and Standards

Deliverables and Standards

Service name	Customer provided	Deliverables and Standards	Delivery cycle
Mouse immunized antibody library screening technical service	Target antigen or/and cell lines	1. Lead antibody sequence: **30-60 lead molecules** with unique sequences 2. Protein: **10 purified preferred antibodies** (0.5 mg~1.5 mg) 3. Reports: Mouse immunized antibody library antibody discovery report; preliminary analysis report of drug development	6-8 weeks After immunization
Alpaca immunized antibody library screening technical service	Target antigen or/and cell lines	1. Lead antibody sequence: **30-60 lead molecules** with unique sequences 2. Protein: **10 purified preferred antibodies** (0.5 mg~1.5 mg) 3. Reports: Mouse immunized antibody library antibody discovery report; preliminary analysis report of drug development	6-8 weeks After immunization
Rabbit immunized antibody library screening technical service	Target antigen or/and cell lines	1. Lead antibody sequence: **30-60 lead molecules** with unique sequences 2. Protein: **10 purified preferred antibodies** (0.5 mg~1.5 mg) 3. Reports: Mouse immunized antibody library antibody discovery report; preliminary analysis report of drug development	6-8 weeks After immunization

Optional additional services

Antibody preparation

High-quality antibody customization
Fast protein reparation

Antibody engineering

Antibody deep human transformation
Antibody ultimate affinity maturation

Pharmacodynamic Analysis

In vitro efficacy analysis of innovative drugs
Animal efficacy and pharmacokinetic analysis

Technical production

Antibody-producing cell line construction
Development of fermentation and purification process
Formulation and quality control method development

THANKS

TOGETHER WE CAN SERVE MORE

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