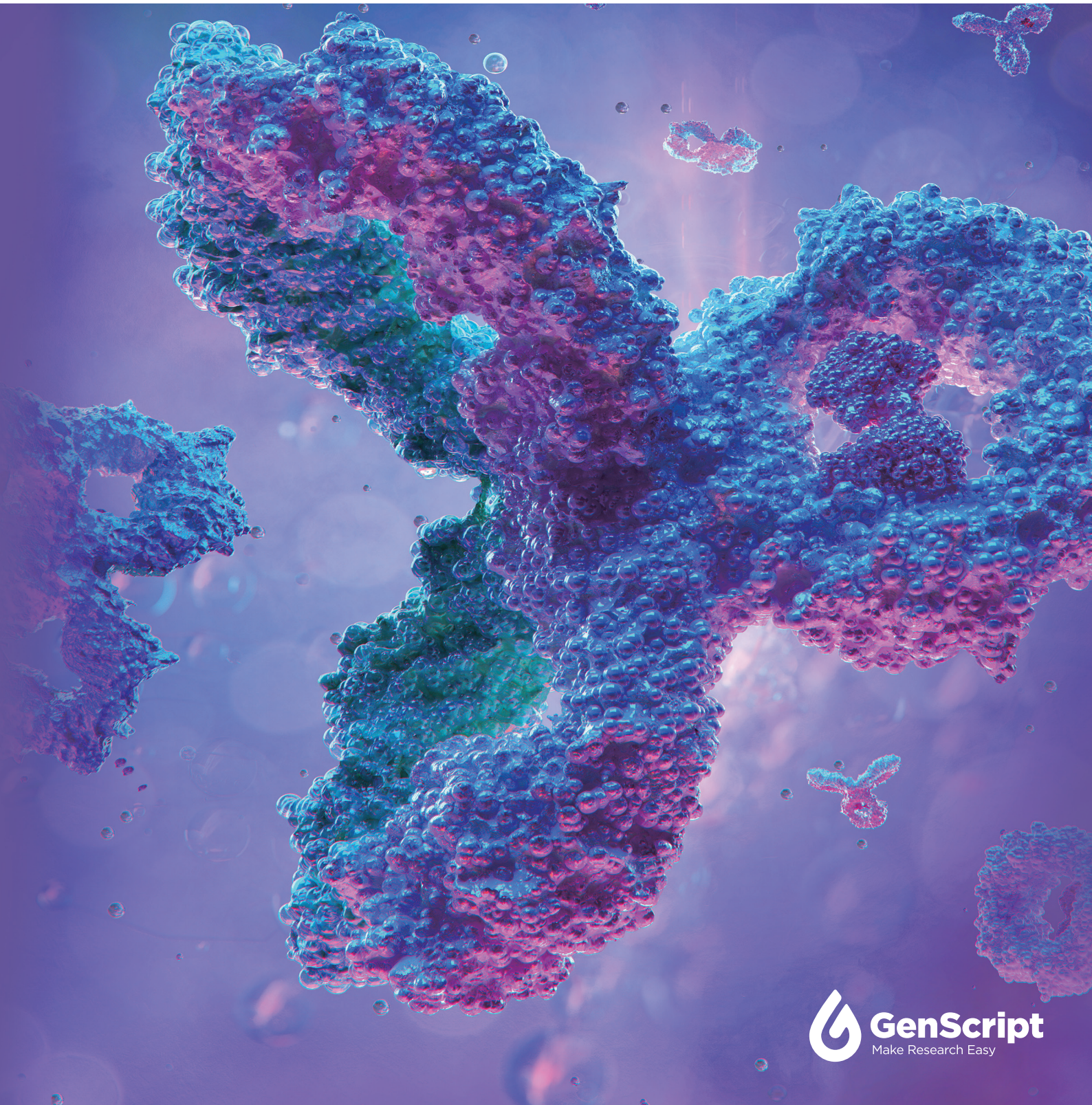
ANTIBODY

Antibody Generation Handbook

www.genscript.com
[2022 edition]



GenScript
Make Research Easy


Make People and Nature
Healthier Through Biotechnology

ABOUT US

GenScript Biotech Corporation (stock code: HK.1548) is a leading global provider of life science research, development and manufacturing services. Rooted in solid gene synthesis technology, GenScript has established four major platforms: life science service and product platform, biomedical contract development manufacture organization (CDMO) platform, cell therapy platform and industrial synthetic biological products platform.

Founded in 2002, GenScript established its R&D and manufacturing headquarters in Nanjing, China in 2004. In 2015, GenScript was listed on the Main Board of the Stock Exchange of Hong Kong, with legal entities in the United States, China, Hong Kong, Japan, Singapore, the Netherlands and Ireland. It operated business in over 100 countries and regions worldwide, providing quality, convenient and reliable services and products for more than 100,000 customers.

As of December 31, 2021, GenScript owned more than 5,200 employees worldwide, with over 40% of them holding a Ph.D. or master's degree. GenScript has a number of intellectual property rights, including more than 180 granted patents and more than 670 patent applications, as well as a high dense technical secrets.

With its mission of “making people and nature healthier with biotechnology”, GenScript is committed to be one of the most trusted biotechnology companies in the world. As of December 31, 2021, GenScript’s services and products have been cited in over 65,600 peer-reviewed international academic periodical articles.

HISTORY & MILESTONES

2002

- **GenScript established in New Jersey, USA**

2004

- Introduced **custom protein and antibody services**
- Established research and production facilities in **Nanjing, China**

2009

- Received investment from **KPCB China/The Balloch Group**

2011

- Established a new R&D and production base in **Nanjing**
- Participated in the **synthetic yeast genome Sc2.0 project** as the only commercial entity selected
- Established **Japanese** subsidiary
- Number of employees reached **1,000**

2013

- **Established Bestzyme** (Industrial Synthetic Bioproducts Division)

2014

- **Established Legend Biotech** (Cell Therapy Division)
- **Awarded the Commissioned Research Institute** Leadership Award

2015

- **Listed on the Hong Kong Stock Exchange** (stock code: HK.1548)

2017

- Legend Biotech and Janssen Biotech entered into a global strategic partnership for **BCMA products**
- **CFDA** accepted Legend Biotech's IND application
- Acquired 100% shares of CustomArray and obtained **chip gene synthesis technology**

2018

- **BCMA Product received IND approval in** China and U.S.
- **BCMA program** progressed well in China and U.S.
- **Biologics CDMO** business unit officially established

2019

- **New GMP Biologics CDMO R&D** Center was in operation
- LCAR-B38M/JNJ-4528 was granted **orphan drug status by the FDA and priority drug status** by the EMA
- **LCAR-B38M /JNJ-4528 U.S. Phase 1b /2 clinical data achieved excellent performance of 100%ORR and 69%CR**

2020

- The Company jointly developed the world's **first neutralization antibody detection kit** with Singapore to combat the epidemic of COVID-19
- Legend Biotech was publicly listed on NASDAQ

2021

- **GenScript ProBio (Biologics CDMO)** became a leading CDMO in gene therapy and cell therapy in China through A round of financing
- Legend Biotech **Cilta-cel** cell therapy product submitted a biologics license application (BLA) to the FDA and expected to be approved by the end of February 2022
- With more than **5,200 employees**, the group distributed life science production capacity in **Singapore and the United States** and cell therapy production capacity in **Belgium** to serve the global market

Content

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Antibody Service Introduction

Our Advantage

Antibodies are essential research tools that enable researchers in different scientific fields to define, identify, locate or quantify protein targets based on antibodies. As an award-winning supplier of customized antibodies, GenScript has successfully delivered over 150,000 antibody projects. GenScript has animal facilities certified by OLAW and AAALAC in which all rodents and rabbits can produce antibodies. From antigen design to customized antibody production, our professional scientists and technical support team will support your R&D projects!

More than 18 years of experience

Delivery of 150,000+ antibody project

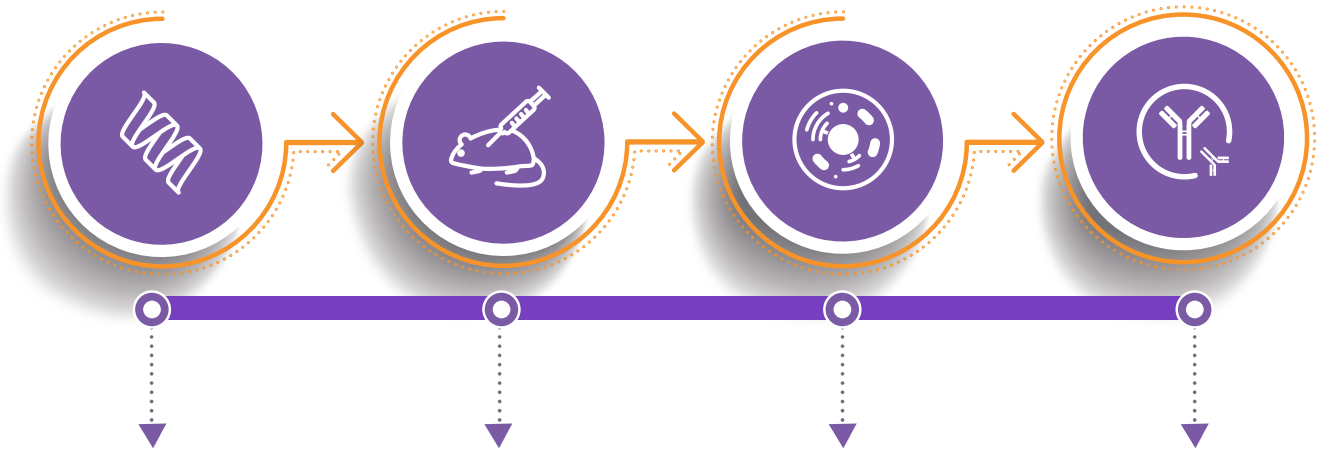
Serve for 5,000+ organizations

Customers in more than 100 countries

- **110,000+** polyclonal antibody preparation project
- **14,000+** monoclonal antibody preparation project
- **400+** therapeutic lead antibody drug development project

Polyclonal antibody project	800-1000 antibodies/month
Monoclonal antibody project	80-100 antibodies/month
Antibody related projects	50 antibodies/month

Antibody Development Process



Antigen Preparation

OptimumAntigen™ antigen design software independently developed by GenScript

High throughput protein expression

Fast delivery of 6 working days with traceability

Customized protein expression

Flexible package content

Cost-effective service

Animal Immunization

Proprietary immune adjuvant library

Immunized animals like rabbits, rats, and mice can be chosen

6,000 m^2 of animal house

Certified by OLAW and AAALAC

Clone Screening

Experienced hybridoma technology platform

B cell cloning platform with high diversity and high success rate

First introduction of Beacon® single B cell sorting platform to facilitate antibody development at full speed

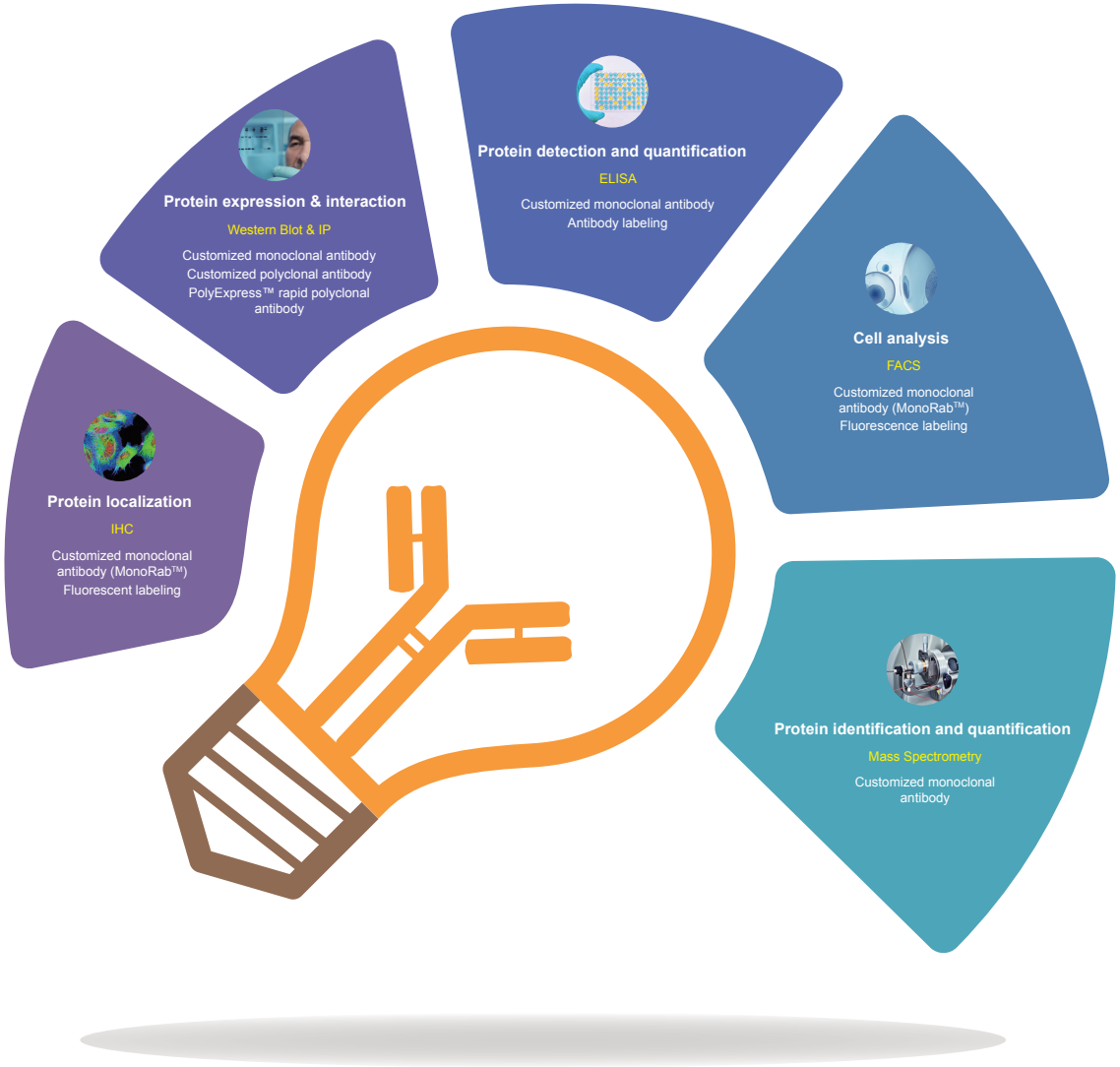
Antibody Purification and Production

Antigen affinity purification and Protein A affinity purification

Antibody production: Shake-flask production and ascites production

Transient and stable recombinant antibody expression

Antibody Application



02

Antibody Service

Antibody Service

Antibody Sequencing

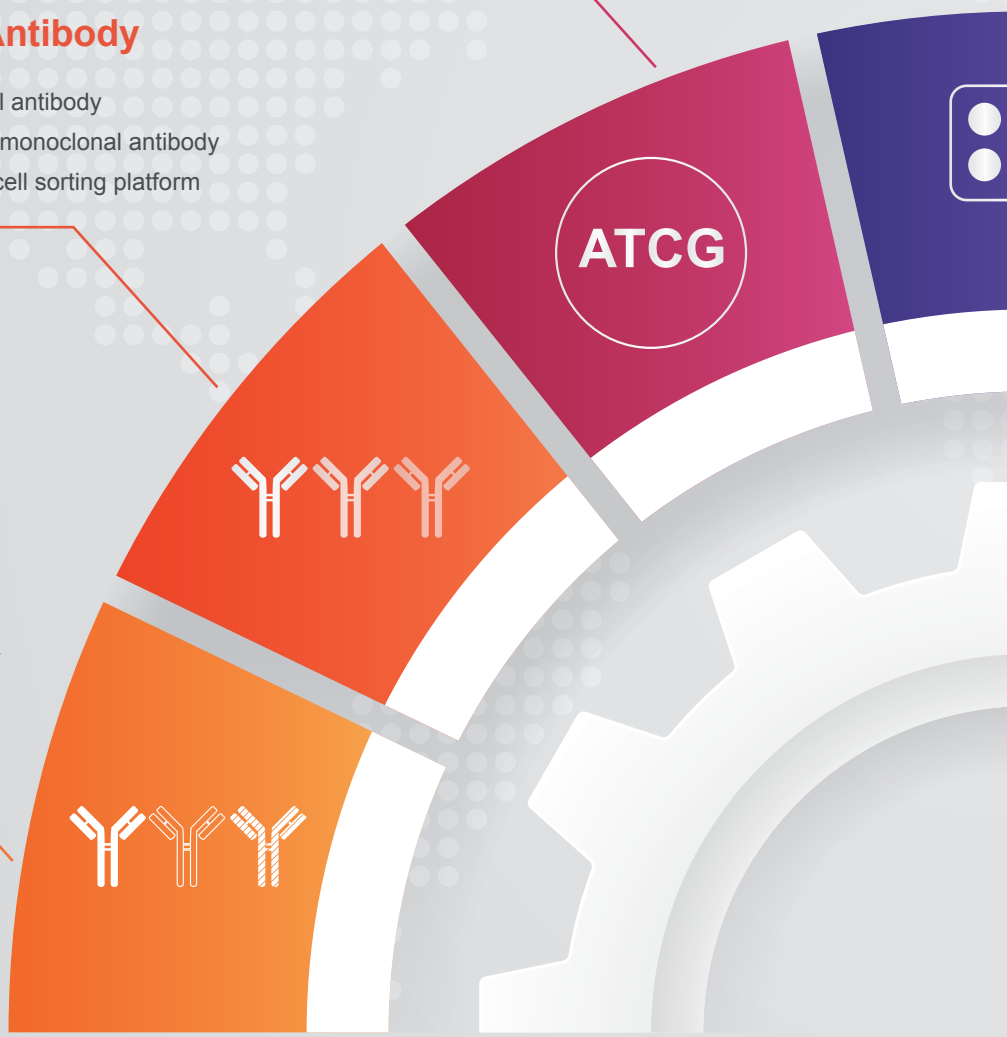
- ✓ Antibody variable region or full-length sequencing
- ✓ High-throughput sequencing of variable regions

Monoclonal Antibody

- ✓ Murine monoclonal antibody
- ✓ MonoRab™ rabbit monoclonal antibody
- ✓ Beacon® single B cell sorting platform

Polyclonal Antibody

- ✓ Rapid polyclonal antibody
- ✓ Customized polyclonal antibody



Kit Development Service

- ✓ ADA ELISA kit development
- ✓ HCP ELISA kit development
- ✓ PK kit development
- ✓ Development of micromagnetic particle based chemiluminescence kit

Phosphorylated antibody

- ✓ Committed antibody ELISA titer ≥ 1:256,000
- ✓ Cross-reaction with non-phosphorylated peptides < 10%

Antibody Production

- ✓ Shake-flask production
- ✓ Ascites production
- ✓ Recombinant antibody preparation

Antibody Modification

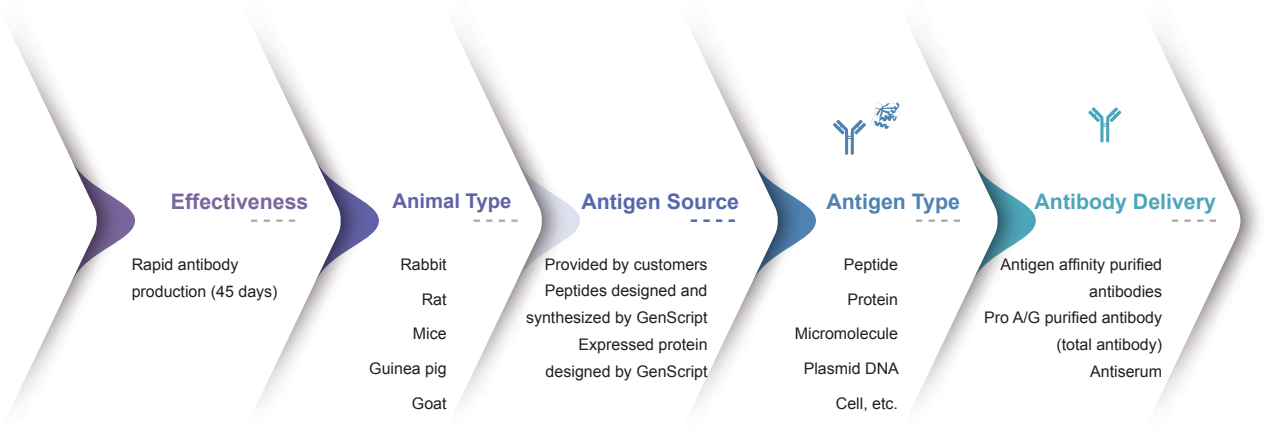
- ✓ Biotin labeling
- ✓ HRP labeling
- ✓ Fluorescein molecular labeling
- ✓ Antibody fragmentation

Polyclonal Antibody

Most antigen molecules have multiple epitopes, and each epitope can stimulate the body to produce a specific antibody. After animals are immunized with antigens, the immune serum was collected and purified to obtain polyclonal antibodies. Polyclonal antibodies recognize multiple epitopes of antigens and detect multi-epitope antigens with good stability.

Polyclonal Antibody Service

- Rapid polyclonal antibody
- Customized polyclonal antibody



Specialized production facilities

- Certified by AAALAC and OLAW
- 6000 m^2 well-equipped animal house with constant temperature and humidity
- The experimental staff are professionally trained and experienced, and operate in strict accordance with SOP
- A complete range of animals, including rabbits, mice, rats, guinea pigs, and SPF level of animals are available.

Rapid polyclonal antibody

The PolyExpress™ Rabbit Polyclonal Antibody Service is a new rapid antibody preparation service from GenScript that combines antigen design software, proprietary optimized adjuvants and rapid immunization protocols. Our service can help customers complete polyclonal antibody production in 45 natural days with a promise of final ELISA titer greater than 1:256,000 and a promise of positive Western Blot results for protein antigens.

Service Advantages

- Fast delivery with **45 days**
- More antibody delivery: **Budget saving and more economy**
- Higher ELISA **titer:** titer >1:256,000, antigen affinity purification about 2.5 mg/ rabbit or 100-300 mg/ goat

We offer proprietary optimization technology, focusing on each step of polyclonal antibody production

Antigen Synthesis

Intelligent design:
More rational antigen design greatly improves the probability of antibody application success

Flexible service:
Customers can provide antigens or provide sequences for antigen synthesis

Immunized Animals

Effective immunity:
Serum titer is 2 gradients higher than that of Freund's adjuvant

Strict production:
Each step of the experiment was carried out in strict accordance with SOP specifications, as the quality is determined by the process

Purified Antibody

Guaranteed ELISA titer:
1:256,000, industry-leading

Guaranteed delivery:
Meet the demand for experimental antibodies

Guaranteed cycle:
Make sure the experiment proceeded on schedule

Service Details

Scientific research makes it so convenient

Antigen → Serum + Antibody

Antigen Preparation
- Peptide or proteins

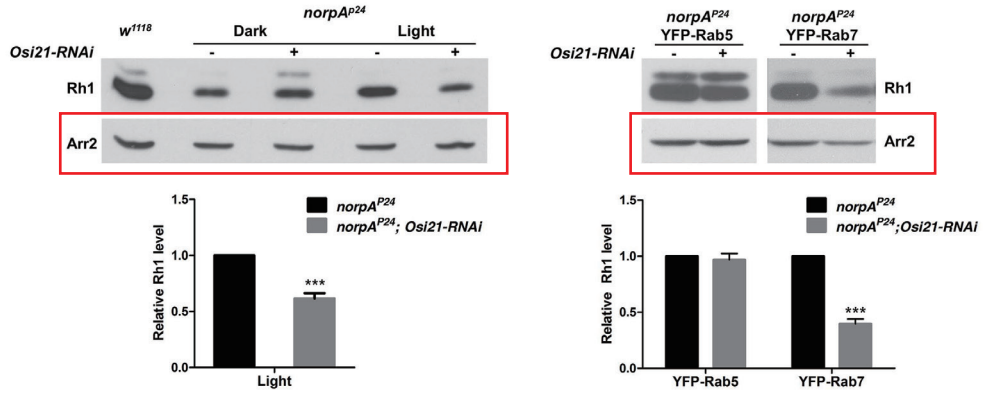
Immunized Animals
- Rabbit

Antibody Purification
- ELISA titer: 1:256,000

Service Name	Antigen	Antibody delivery*	Antigen Delivery	Delivery Standard	Cycle
Cat SC1676	1 Protein	2 purified polyclonal antibodies (1.5-6 mg/rabbit)	200 μg antigenic protein	ELISA titer 1:256,000 WB positive	10-11 weeks
Cat SC1180	1 peptide	1 purified polyclonal antibody ≥ 2 mg	Antigenic peptide ≥ 2 mg	ELISA titer 1:256,000	45 days

* Hint: Selecting proteins and multiple peptides designed based on different regions of proteins as antigens has a higher success rate of obtaining antibodies matching antigen proteins.

Case:



GenScript provides Arr2 antibody for Western Blot to detect the effect of Osi21 knockdown on Rh1 and Arr2.

Customized Polyclonal Antibody

GenScript provides one-stop customized polyclonal antibodies solutions, from antigen design, antigen preparation, animal immunization to antibody purification and QC testing (ELISA test, etc.), offering you with a full range of customized antibody services.

Our polyclonal antibody service covers rabbit, mouse, rat and other species. Antibody tests can be carried out in AAALAC and OLAW international certified laboratories. We advocate humane and scientific treatment of animals and ensure the quality of animal experiments.

Service Details

Antigen Source	Antibody delivery (different ways of antibody purification)	Amount of Antibody Delivery		Delivery Standard (ELISA)	Delivery Cycle	
		Peptide Antigen	Protein Antigen		Peptide Antigen	Protein Antigen
GenScript synthetic antigen (peptide/protein)	Protein A affinity purified antibodies	200-500 mg	Welcome your enquiry	1:256,000	13 weeks	Welcome your enquiry
	Antigen affinity purified antibodies (phosphorylated polyclonal antibody)	1-30 mg (0.1 mg "Pan" antibody)		1:256,000	16 weeks	
Antigen (peptide/protein) provided by customer	Antigen affinity purified antibodies	3-50 mg		1:256,000	11 weeks	
	Protein A affinity purified antibodies	200-500 mg		1:256,000	11 weeks	

Pan antibody: Most protein families have different members. Different members may use the same motif or sequence, but different proteins have different functions. Antibodies that recognize different members of the same protein family are called Pan antibodies.

Monoclonal Antibody

Monoclonal antibodies are highly homogeneous antibodies produced by a single B cell and directed only against a specific antigenic epitope. Hybridoma technology, single B cell screening technology and phage display technology are commonly used to obtain monoclonal antibodies. Hybridoma technology refers to the fusion of B cells that produce specific antibodies and proliferative myeloma cells to form cells with antibody-secreting function and permanent proliferation, so as to obtain monoclonal antibodies. Single B cell screening technology refers to the direct screening of IgG secreting single B cells from the animal B cell antibody library with advanced high-throughput DNA sequencing technology, such as the Next Generation Sequence (NGS), so as to obtain natural paired antibodies with high specificity and affinity.

Murine hybridoma

Antibody Source	Cells of the spleen or lymph node
Delivery of Products	Cells and antibodies
Overall Cycle	About 14 weeks[1]
Price	Low

B Cell Cloning Platform

Antibody Source	Memory B cell
Delivery of Products	Antibody and its sequence
Overall Cycle	About 10 weeks[2]
Price	Medium

Beacon® single B cell platform

Antibody Source	Mouse: Cells of the spleen or lymph node Rabbit: Memory B cell
Delivery of Products	Antibody and its sequence
Overall Cycle	About 8 weeks[3]
Price	High

Calculation standard:

1: non-difficult target, rapid immunization, no additional immunization required, one step antibody discovery, hybridoma antibody production and purification

2: non-difficult target, rapid immunization, no additional immunization required, one step antibody discovery, sequencing, antibody production and purification

3: non-difficult target, rapid immunization, no additional immunization required, a chip, sequencing, antibody production and purification

Service Features

- Rapid preparation of monoclonal antibodies and customized monoclonal antibodies
- Three major platforms: hybridoma platform, B cell cloning platform, Beacon® single B cell sorting platform
- High quality, the antibody titer up to 1:512,000

Guaranteed Murine Monoclonal Antibody

To meet customer needs for monoclonal antibodies, GenScript rapid preparation service for monoclonal antibodies allows clone delivery within 10 weeks. We guarantee to provide customers with multiple positive clones and purified antibodies with high affinity through OptimunAntigen™ antigen design software and independently developed adjuvants. For this service, we will guarantee the delivered antibodies with positive WB results with immunogens.

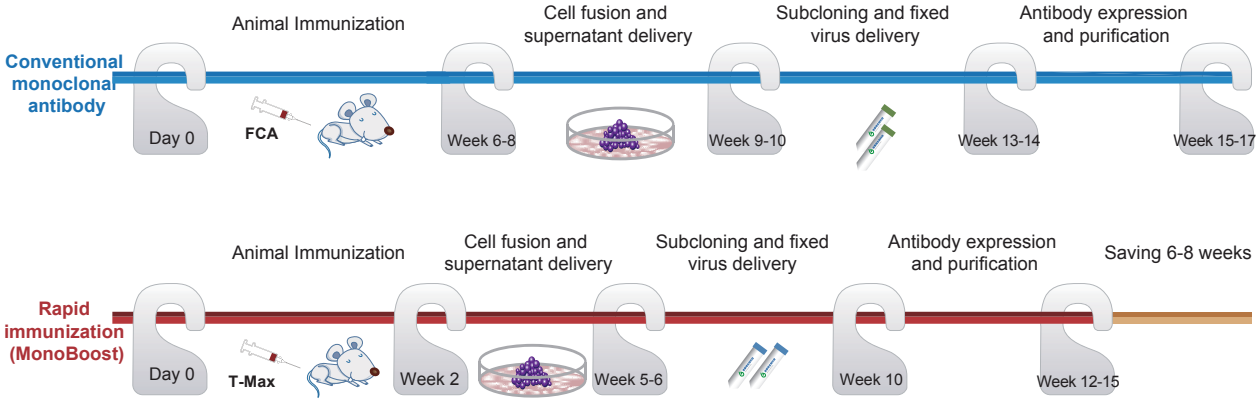
Service Advantages

Fast delivery of supernatant of a mother clone with **45 days**

High quality antibody

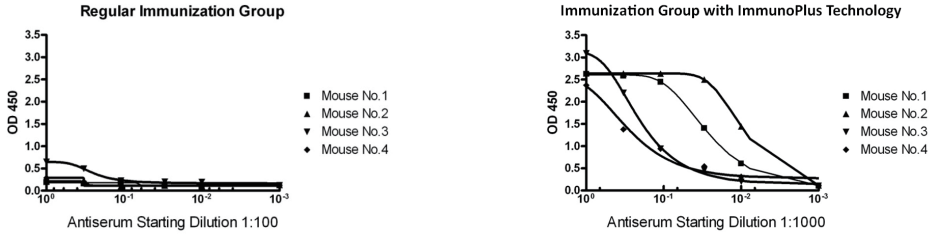
Production **specification**

Service Details

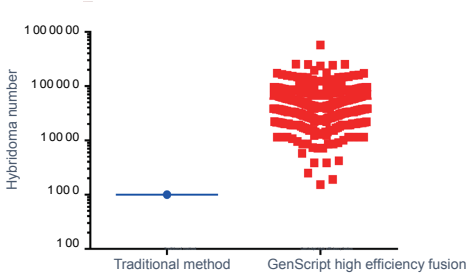


Protein Antigen Package	Guarantee	Starting Material	Number of immunized animals	Customer Screening	Deliverables	First Delivery
Accelerated By MonoBoost (SC2129)	At least 1 WB positive clone for immunogen ELISA ≥ 1:64,000	GenScript generated antigen Customer provided antigen	3	30 ELISA positive supernatants for initial evaluation by client	1 WB positive clone for immunogen Up to 5 hybridoma cell lines (2 vials/clone) 2 mg purified mAb from 1 clone 0.2 mg purified antigen (if GenScript provides)	8 wks
Classic (SC2128)	At least 1 clone that is WB positive for immunogen ELISA ≥ 1:64,000	GenScript generated antigen Customer provided antigen	3	20 ELISA positive supernatants for initial evaluation by client	1 WB positive clone for immunogen Up to 5 hybridoma cell lines (2 vials/clone) 2 mg purified mAb from 1 clone 0.2 mg purified antigen (if GenScript provides)	18-25 wks

Cases:



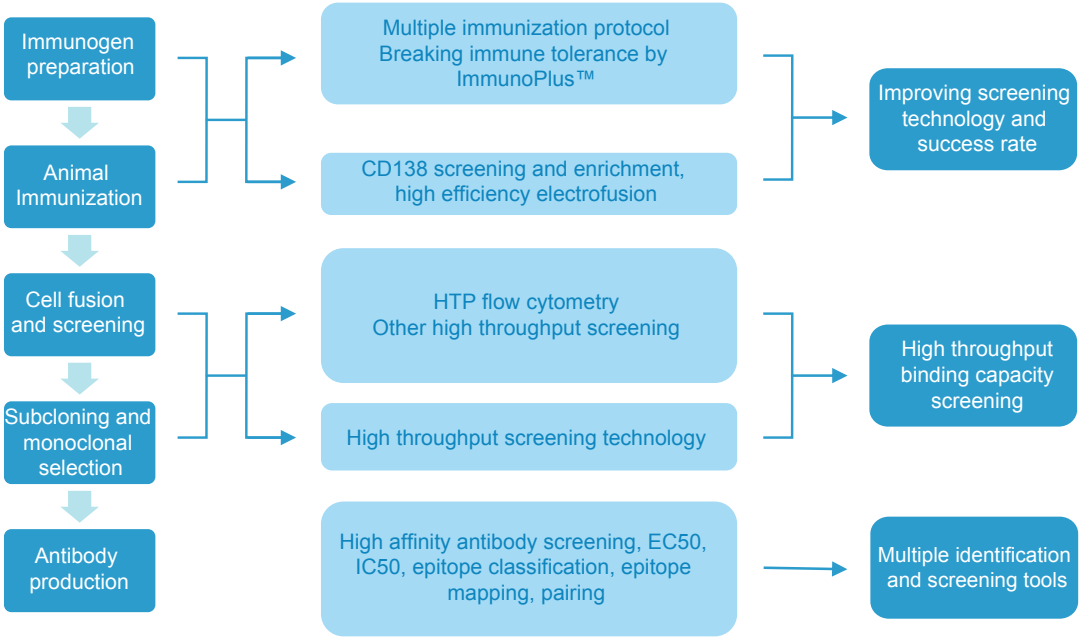
ImmunoPlus™ can greatly improve the immune response of antigens with 96% homology.



By comparing the traditional method (left) with the GenScript high-efficiency capacitor method (right), the GenScript fusion method has higher electrofusion efficiency, which effectively enhances the library capacity and improves the success rate of positive antibody screening.

Customized murine monoclonal antibody

GenScript offers a comprehensive customized service for murine monoclonal antibodies including the animal species, number of immunized animals, immunization method (rapid and traditional), screening method (ELISA or FAC), and other stages to fully meet your needs for antibody downstream applications. GenScript can also assist you in scaling up production when the developed antibody is on demand.



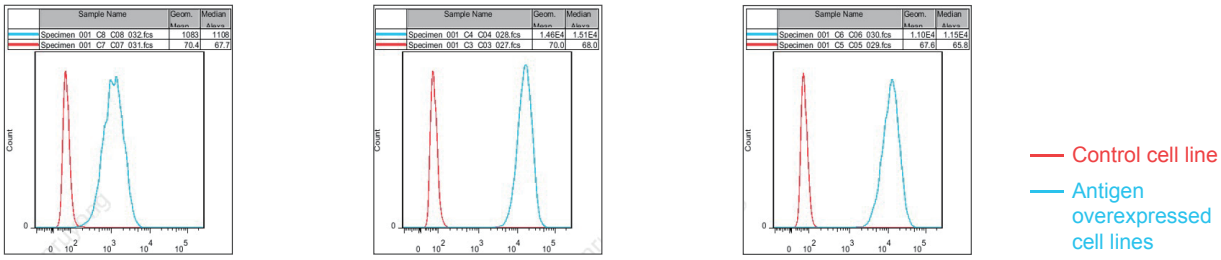
Service Details

Phase	Package selection	Delivery	Cycle
Phase I Immunization	• MonoBoost or traditional immunization methods (increase by 6-8 weeks) • ImmunoPlus™ technology • DNA immunization • Animals Balb/ C mice, C57BL6 mice or rats • Number of immunized animal	test report	8-10 weeks (conventional immunization) 2-4 weeks (MonoBoost™ immunization)
Phase II Cell fusion and screening	• Indirect ELISA or Westen Blot assay • Capture-ELISA • Competitive ELISA • Flow cytometry • Additional monoclonal screening	2 mL supernatant/strain	4-6 weeks
Phase III Subclonal screening and amplification	• Subclonal screening • Cryopreservation • Cell banking • Roller bottle production • Antibody purification	Hybridoma cell lines 5 mL subclonal supernatant Purified antibody	6-8 weeks

Case 1: Preparation of murine monoclonal antibody for FACs assay

Antigen: Peptide **Application: FACS**

<Serum titer> <Clone 1> <Clone 2>

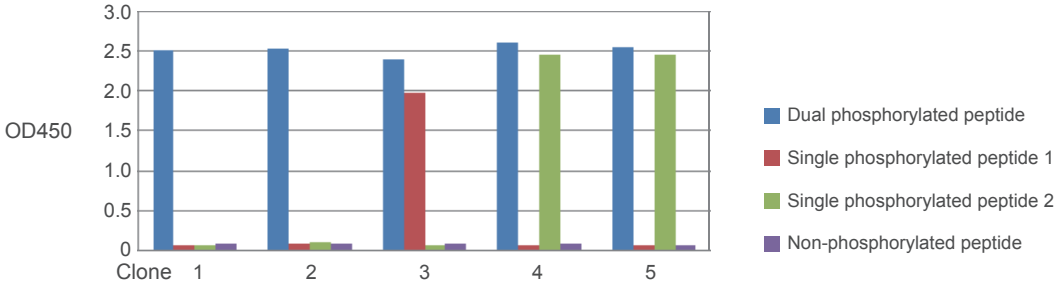


- Difficulty 1: The homology of immunogen is 100% with mouse
- Difficulty 2: Immunization with peptides, for FACs application

Proprietary *ImmunoPlus*™ technology to break immune tolerance and produce murine monoclonal antibodies with high homologous antigens

Case 2: Preparation of murine monoclonal antibodies that recognize specific phosphorylated sites

Immunogen: Dual phosphorylated peptide Test material: 4 different peptides Immunization protocol: MonoBoost™



- Clone 1 & 2: Only recognized dual phosphorylated peptide
- Clone 3: Recognized dual phosphorylated peptide and single phosphorylated peptide 1
- Clone 4 & 5: Recognized dual phosphorylated peptide and single phosphorylated peptide 2

***MonoBoost*™ Immunization improves the success rate of murine monoclonal antibody o recognize the PTM sites**

Case 3: Improvement of hybridoma positive rate by plasma cell enrichment

Project	Fusion with initiating cells	Number of plate	Number of positive clone	Positive rate
Project I: Conventional peptide immunization	CD138 + spleen cells	5	58	12%
	Spleen cells	5	5	1%
Project II: MonoBoost™ protein immunization	CD138 + lymph node cells	10	826	86%
	Lymph node cells	10	120	13%
Project III: MonoBoost™ protein immunization	CD138 + lymph node cells	7	190	28%
	Lymph node cells	7	33	5%

CD138+ enrichment *increases the positive rate of hybridoma*, which is suitable for difficult projects and projects with high diversity requirements

MonoRab™ rabbit monoclonal antibody

Rabbit possess a unique B cell maturation and development mechanism, which is more likely to produce strong immune response to small molecules or weak immunogenicity antigens. Therefore, it is easier to obtain antibodies against specific modified sites, saccharides, haptens and small molecules through rabbit monoclonal antibody technology. With its unique advantages, the rabbit monoclonal antibody has been widely used in the field of life science, including basic research, diagnosis and treatment. GenScript provides high affinity, specificity and diversity of rabbit monoclonal antibody services, covering two R&D platforms (B cell cloning and single B cell sorting), to assist you in the R&D process at full speed!

Service Features

High affinity

Rabbit B cells can produce picomolar dissociation coefficient (i.e., high affinity) during maturation, which is 10-100 times higher than rodents

High specificity

Rabbit monoclonal antibodies have stronger ability to distinguish similar epitopes and better specificity

Strong stability

The unique IgG structure of rabbits enhances the stability of the antibody, which is better than that by rodents

Diversity

Compared with mice, rabbits have higher recognition coverage to antigens and produce antibodies with higher diversity

Customized rabbit monoclonal antibody

GenScript MonoRab™ rabbit monoclonal antibody service has a R&D platform with **B-cell cloning and single B-cell sorting** as its core. Combined with the advantages of rabbit autoimmunity, we can provide high quality rabbit monoclonal antibodies for a variety of difficult projects for your multiple applications.

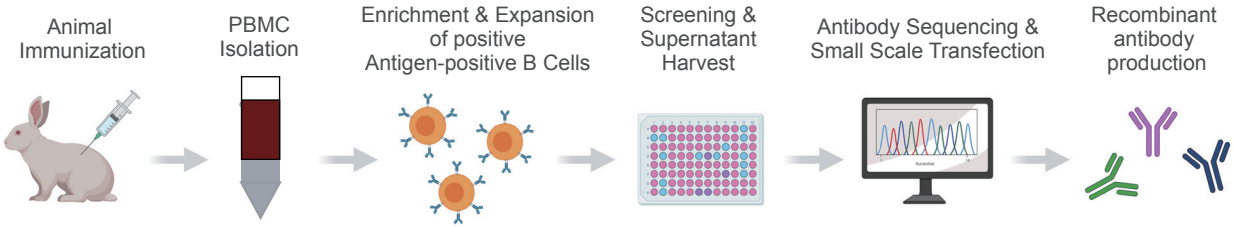
An average delivery of 50 clones

Cost-effective

Multiple applications

Service Details (B cell cloning as an example)

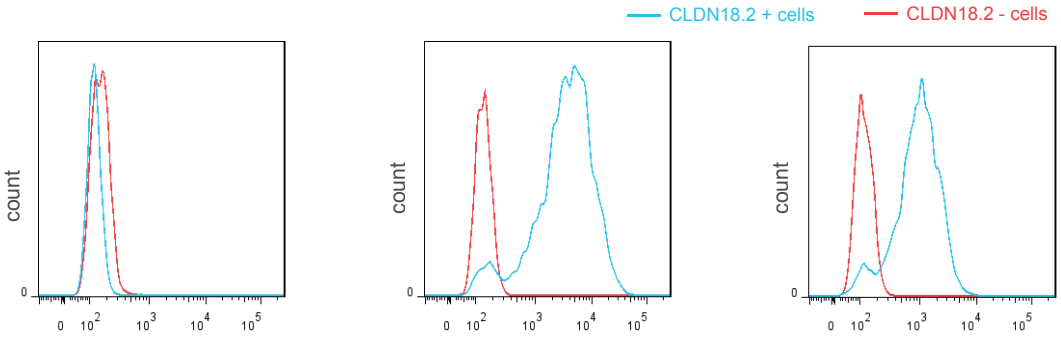
Phase	Description	Timeline	Deliverables
I) Animal Immunization	4 rabbits (express or conventional immunization)	Express: 5 - 6 weeks Conventional: 8 - 10 weeks	• Antiserum report • Small scale pAb purification (optional)
II) B Cell Clonin and Screening	1 round of B cell cloning	3 weeks	• Positive clones report • 1 ml B cell supernatant delivery, up to 50 clones (optional) • Additional screening and increased clone number delivery options available
III) Antibody Sequencing & Small Scale Transfection	Sequencing of 5 customer-selected clones, followed by small scale rAb expression	3 weeks	• Sequence reports • Up to 5 rAb supernatants (2 ml), or purified rAb (0.1 mg) • Additional sequencing and supernatant delivery options available
IV) Recombinant Antibody Production	Scalable expression of selected recombinant rabbit mAbs	3 weeks	• Sequencing report • Purified antibody



Fast with 11 weeks

Case 1:

Combined immunization with multiple transmembrane proteins



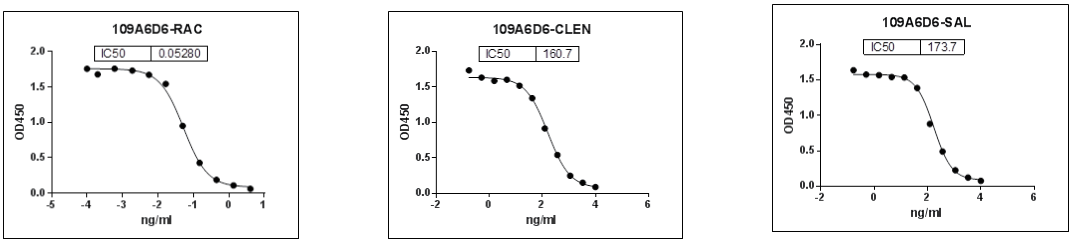
[Pre-immunized Serum] [Positive reference antibody] [4790# Rabbit immunized serum]

The positive reference antibody was used to compare the immunized results, which showed that the rabbit immunized serum obtained by our combined immunization scheme was equivalent to the positive reference antibody.

Case 2:

High specificity of monoclonal antibody against small molecule

Target small molecule | Analogues 1 | Analogues 2

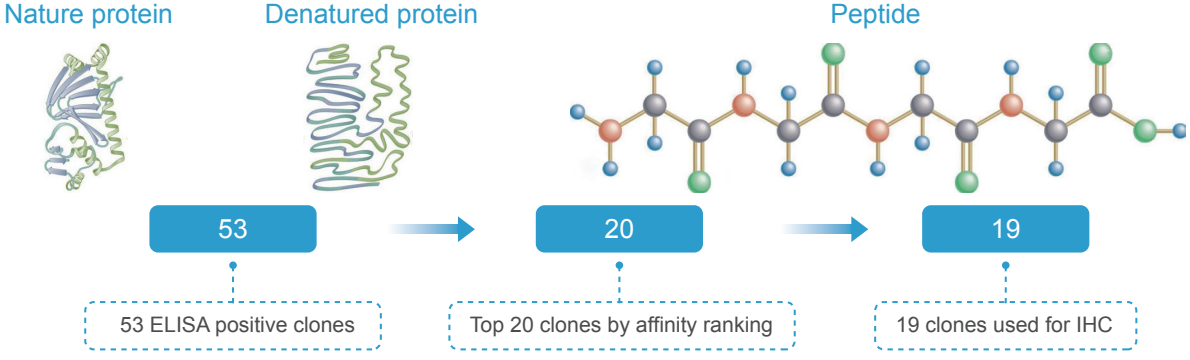


Affinity: $K_D \approx 10^{-11}$M | Cross reaction: < 0.0329% | Cross reaction: < 0.0304%

The antibody affinity of the target molecule reaches 10^{-11}, and its cross-reactivity with analogues is low.

Case 3:

Recognized different antigen conformations for application in immunohistochemistry



Single B Cell Sorting Platform

GenScript introduced Beacon® in early 2020 and became the first antibody R&D service company using Beacon® in Asia Pacific. Beacon® is a single-cell sorting device based on integrated microfluidic technology, signal detection system and photoinduced two-dimensional electrophoresis. The screening scope of single B cell sorting platform covers more than 90% **B cells**; in terms of screening time, the screening with Beacon® is completed in **only one day**, saving **12 weeks** compared with hybridoma. Combined with the above advantages, the single B cell sorting platform is a superior choice for large-scale B cell screening.

Service Advantages

Captured target clone with high probability

Faster screening time

Automated screening process

Higher diversity and affinity

Average cell loading number per chip > 10,000

Average monoclones per chip > 200

Screening: 3-5 rounds/day

High throughput: 4 chips screened simultaneously

Multiple antigen binding screening

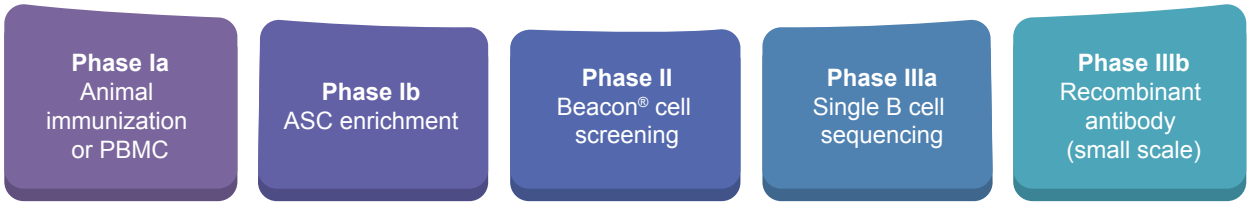
Cell functional screening

Other customized protocols

Project management team (Ph.D.)

Project experience: > 40

Service Details



Cycle: 4-6 weeks	Cycle: 5 days (rabbit) / 5 hours (mouse)	*On Beacon®* Cycle:1 day	Cycle:7 days	Cycle:4 days

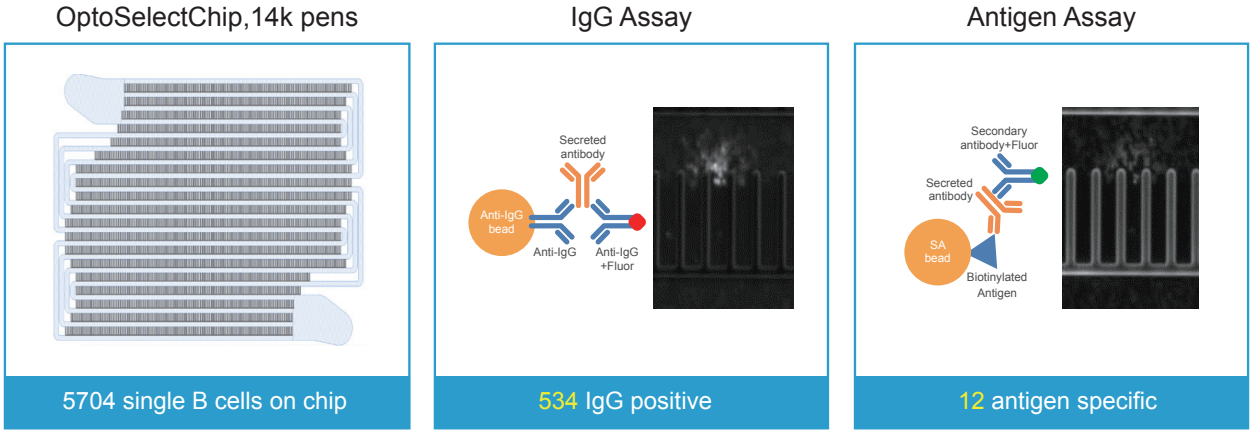
Three Phases of Single B Cell Screening:

Phase I: Animal immunization, serum detection and enrichment of ASC cells;

Phase II: Beacon® cell screening, including ASC cell introduction, chip assay and positive cell export;

Phase III: Single B cell sequencing, small-scale antibody expression.

Case 1:

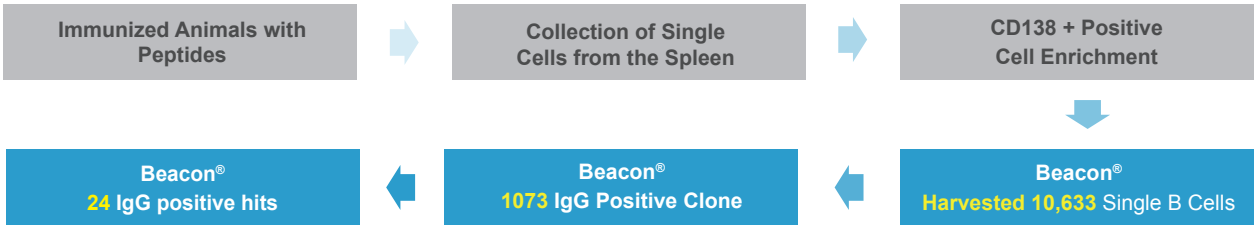


(A) (B) (C)

Antibodies against transmembrane protein (murine)

(A) 5704 B cells were screened on the chip with Beacon®; (B) 534 IgG-positive clones were obtained by chip screening; (C) 12 positive clones were obtained by antigen screening. In other cases, 21 antigen-specific clones were harvested for glycosylated transmembrane proteins.

Case 2:



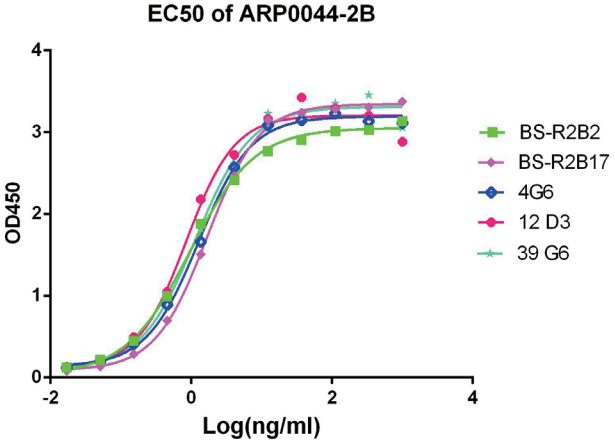
Peptide Antigen (murine)

Peptides were applied as the antigen for animal immunization, then CD138 + cells were collected from spleen cells, then 1073 IgG positive clones were harvested by Beacon® single B cell screening, and 24 antigen positive clones were obtained by final antigen screening.

In addition to the above cases, GenScript also has other cases of monoclonal antibodies against COVID-19 N and S proteins. The immunized animals include rabbits and mice, and the antigens include general proteins, peptides, glycosylated transmembrane proteins, and GPI-linked glycoproteins.

Case 3:

High affinity and diversity of rabbit monoclonal antibodies against SARS-CoV-2 S protein



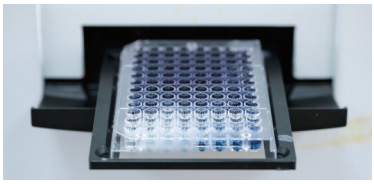
Epitope 1	BS-R2B12	BS-R2B16	BS-R2B30	
Epitope 2	BS-R2B17	BS-R2B27	BS-R2B50	
Epitope 3	BS-R2B2	4G6	12D3	39G6
Epitope 4	BS-R1B8			

Clone Name	BS-R2B1	BS-R2B2	BS-R2B12	BS-R2B16	BS-R2B17	BS-R2B27	BS-R2B30	BS-R2B50	BS-R1B8	4G6	12D3	39G6
EC50 (ng/ml)	9.57	0.96	1.56	4.28	1.68	12.74	0.96	20.12	2.67	1.28	0.90	1.17

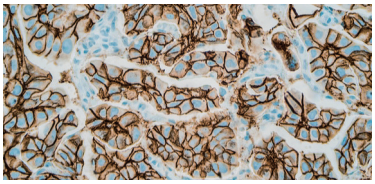
Applications

Basic Research

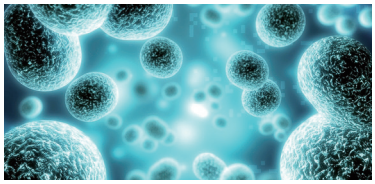
The monoclonal antibody is widely used in the field of basic scientific research. It can be used to study the relationship between the antigen structure and its function at different levels, like molecular, cell and tissue, and then to clarify its mechanism theoretically. For example, fluorescent labeled monoclonal antibodies can be used as probes to conveniently determine the location and distribution of biomacromolecules bound to them in cells.



ELISA



Immunohistochemical

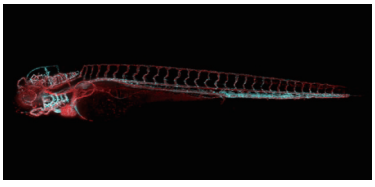


FACS

Diagnostics

***In vitro*:** Various *In Vitro diagnostic* (IVD) assays, such as immunohistochemistry, ELISA and radio immunoassays (RI), are based on the principle of antigen and antibody binding.

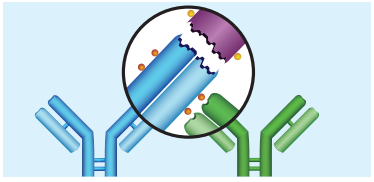
***In vivo*:** Visualization of monoclonal antibodies can be achieved by a variety of approaches; The most common approach is to label monoclonal antibodies with radioactive or fluorescent molecules to help tracking monoclonal antibodies in the body and quantifying the results.

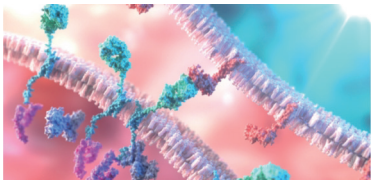


Treatment

Anti-idiotype antibody: Pharmacokinetic (PK) is used to measure the absorption and excretion rates, distribution, and half-life of a candidate small molecule or monoclonal antibody treatment. The results help to determine the optimal dose and toxicity of the therapeutic molecule. Therefore, anti-idiotypic antibodies require sufficient sensitivity to capture antibody targets either in free type, binding type or both two types in biological fluid samples.

Monoclonal antibody treatment: The high specificity and sensitivity of antibody to target antigen can both improve the safety and reduce the toxicity, highly specific antibodies avoid off-target binding and provide safety guarantee; highly sensitive antibodies require low doses of administration, further reducing the toxicity.





Antibody Sequencing

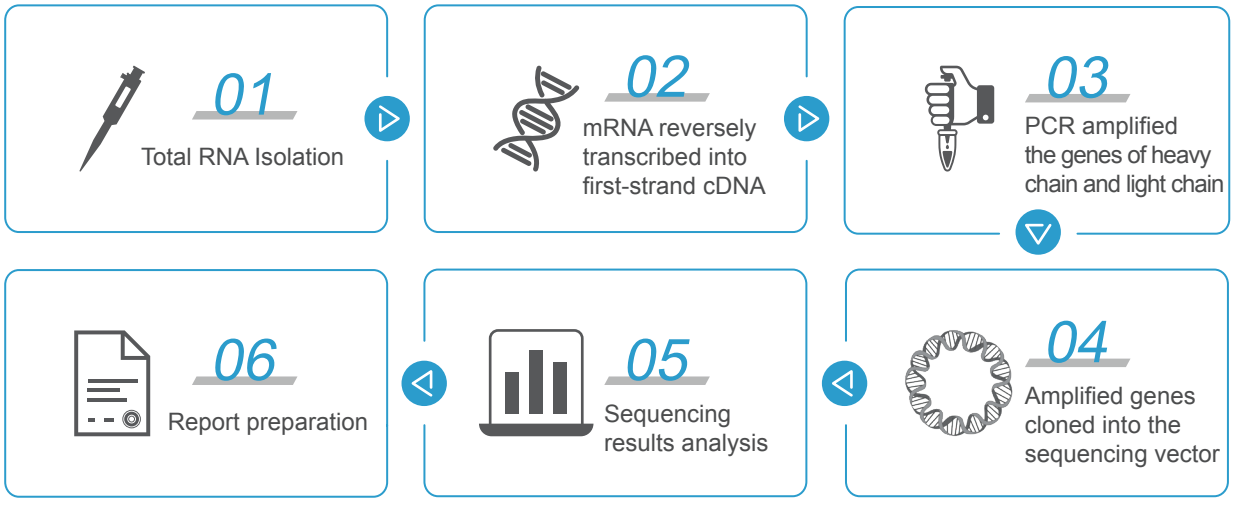
The antibody sequences are obtained by sequencing the hybridoma cells, which can avoid the inability of hybridoma with negative transformation in long-term culture. Antibody sequencing plays an important role in antibody drug development and antibody engineering.

GenScript provides monoclonal antibody sequencing services based on advanced antibody discovery platforms. Monoclonal antibody sequencing includes conventional/sanger sequencing and high-throughput sequencing.

Service Advantages

- **High accuracy**
- **Rich experience**
- **Fast delivery within 10 working days**
- **High throughput**

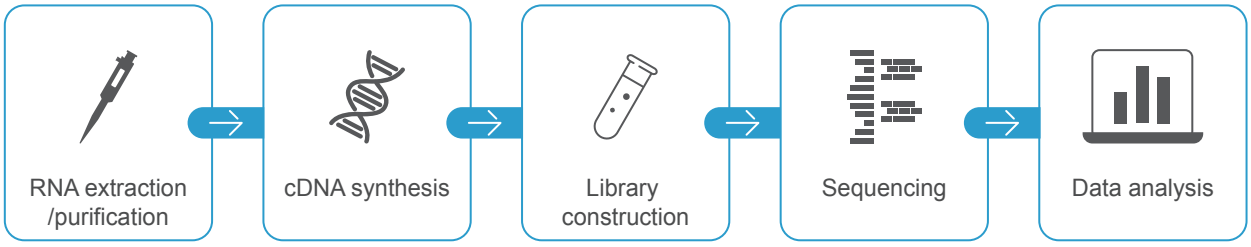
Conventional Monoclonal Antibody Sequencing



Service Content	Delivery Time	Deliverables
Sequencing of Variable Regions	Fast delivery within 10 working days	Final sequence report Sequence raw data Heavy chain and light chain sequence comparison file# Plasmids containing antibody fragments sequence# Expression vectors containing heavy and light chains#*
Full-length antibody sequencing service	Fast delivery within 10 working days	

* The latest upgrade of monoclonal antibody sequencing service offers preferential price, speed improvement and promises no charge for inaccurate sequencing
# Delivery of optional items requires additional fees
#* Expression vectors can be selected from GenScript list or provided by the customer

High Throughput Sequencing



	Number of Clones	Platform	Starting materials	Delivery	Cycle
Variable regions	21-50	NGS (Miseq)	• Hybridoma: Cell suspension Cell precipitation/RNA/cDNA • Single B cell	• Final sequence report • Expression vectors containing heavy and light chains# **(additional charge and 1 week lead time required)**	10 working days
	51-100				
	101-200				
	201-300				

Deliverables are not a default and can be provided on your request at an additional cost

Category	Additional service	Delivery	Cycle
High throughput sequencing	Light and heavy chain expression vectors	10 μg research level expression vector	1 week
	1 mg recombinant antibody	Guaranteed delivery	18 working days
	Antigen binding confirmation (contained in 1 mg recombinant antibody)	Laboratory report	5 working days
	Subtype testing	Subtype testing report	1 working day
Conventional monoclonal antibody sequencing	Sequencing plasmid and QC file	Plasmid and QC file	/
	Subcloned into expression vectors	10 μg research level expression vector	1 week
	Light and heavy chain comparison	Comparison file	/
	Sequencing raw data	File in ab1 format	/
	1 mg recombinant antibody	Guaranteed delivery	18 working days
	Antigen binding confirmation (contained in 1 mg recombinant antibody)	Laboratory report	5 working days
	Subtype testing	Subtype testing report	1 working day

Cases:

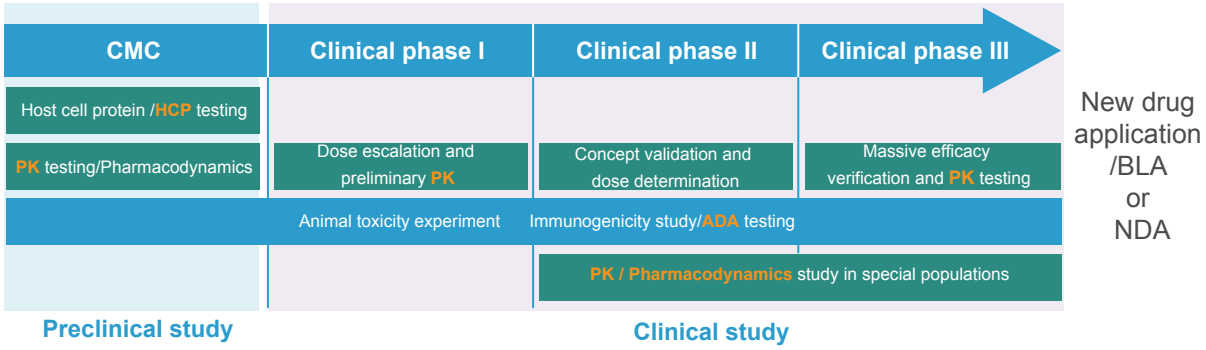
Type	Subtype	
Mice	IgG, IgM, IgA	Kappa/Lambda
Rat	IgG, IgM	Kappa/Lambda
Human	IgG, IgM, IgE	Kappa/Lambda
Rabbit	IgG, IgM	Kappa/Lambda
Hamster	IgG	Kappa/Lambda
Macaque	IgG	Kappa/Lambda
Alpaca	IgG	Kappa/Lambda
Transgenic animals	IgG	Kappa/Lambda

Kit Development

With the development of biomedical research and the innovation of biotechnology, antibody drugs and other biological agents have made remarkable progress in the treatment in a variety of diseases (including cancer, autoimmune diseases, and inflammation). Therefore, the immunogenicity detection, pharmacokinetic detection and host cell protein residue detection of biopharmaceutical clinical research is particularly important.

Leveraging advanced scientific research concept and strong development capacity, GenScript provides customers with efficient and fast immunodetection kit development services based on advanced technology and rich experience in antigen preparation, protein expression and purification, antibody preparation and various immunoassays,.

The kit is used throughout the drug development and IND filling process



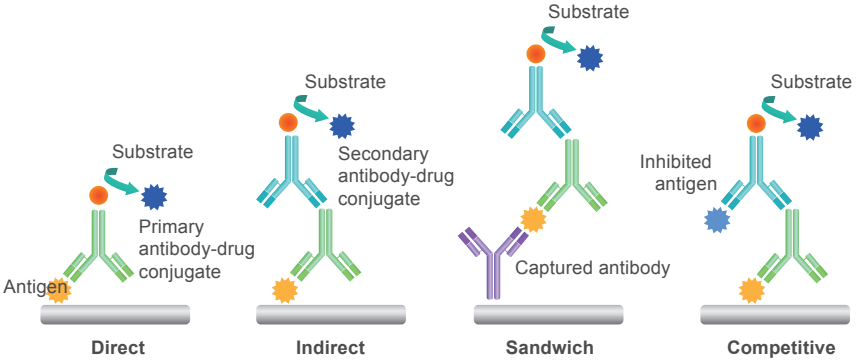
Service Advantages

Fast Turnaround time
9-week fast production from antibody to kit

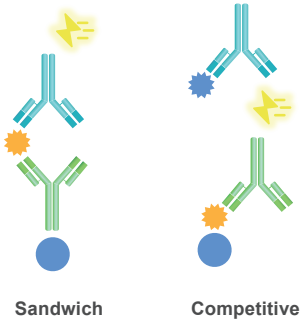
Abundant production experience
>17 years of antibody development experience

Released above FDA standards
Precision: C.V.<10%

One stop solution from research grade to GMP
Meet the needs of different applications



[ELISA kit]



[Micro magnetic particle chemiluminescence kit]

GenScript provides excellent ELISA kit development platforms and experienced antigen and antibody development systems, which can provide the ELISA kit development empowered by micro magnetic particle chemiluminescence technology.

	ELISA	Micro magnetic particle chemiluminescence
Testing signal	Chromogenic product	Optical signal
Sensitivity	pg-ng/mL	pg/mL
Linear coverage	$<10^4$	$>10^4$
Device requirement	Low	High
Application	Preferred in research	Preferred in industry
Timeline	9-19 weeks	

Service Details

Steps	Original materials	Deliverables	Cycle
Prove of concept	Preliminary determination of methods Preliminary establishment of standard curve	Periodic report	1-2 weeks
Assay development	Optimization of kit reaction conditions and parameters Determination of standard curve Stability evaluation, etc.	Periodic report	3-4 weeks
Assay validation	Determination of sensitivity Measurement of intra-batch difference and inter-batch difference Determination of recovery range Determination of samples, etc.	Periodic report and 10 kits	3-4 weeks
Kit manufacturing*	Provided kit	Kit	2-3 weeks

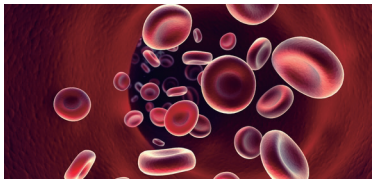
* For more kits, you can order with an MOQ of 5 pcs.

Kit application

Agriculture diagnosis

Quantitative detection of pesticide residues and drugs

Food safety inspection



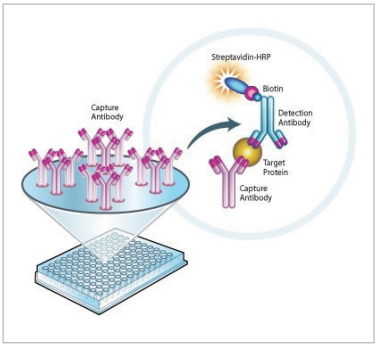
Pharmacokinetic study

Diagnosis of animal and plant diseases

Immunogenicity analysis

Case 1:

PK kit development



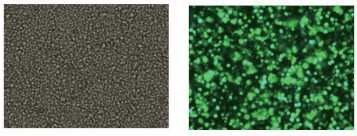
PK kits developed by GenScript meet or exceed FDA requirements

Parameter	Quantity	FDA requirement
Testing range	0.5-24 ng/mL	-
Sensitivity	0.25 ng/mL	-
Precision - intra-batch difference	≤5%	≤20%
Precision - inter-batch difference	≤5%	≤20%
Recovery	80-120%	80-120%
Selectivity	80%	80%
Specificity	Undetected	-
Matrix	Human serum	-

Case 2:

HCP Kit Development

CHO HCP detected by sandwich ELISA



HCP kits developed by GenScript exceed FDA requirements

Parameter	Quantity	FDA standard
Testing range	1-100 ng/mL	-
Sensitivity	0.608 ng/mL	-
Precision - intra-batch difference	≤5%	≤20%
Precision - inter-batch difference	≤10%	≤20%
Recovery	85-115%	70-130%
Specificity	Drug	-
Matrix	Solution for injection	-

Case 3:

HCP Kit Development

HMW
HCP with molecular weight greater than 30 kD

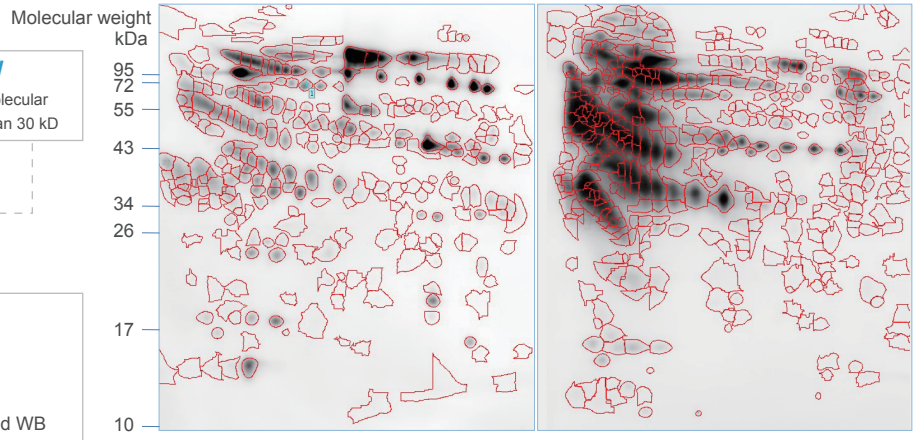
LMW
HCP with molecular weight less than 30 kD

Respectively for antibody preparation

Purified antibody

1. Antibody purity: > 90%
2. ELISA titer > 1:64,000
3. Recognition coverage of PAGE and WB detection > 50%

Preparation of HCP antibody: The preparation was conducted respectively according to molecular weight, resulting in high antibody coverage



Phosphorylated antibody

GenScript provides customized phosphorylated protein-specific polyclonal antibodies and monoclonal antibodies with a delivery success rate of more than 95%. Post-transcriptional modification of proteins, especially phosphorylation of proteins, is involved in almost all cellular activities in the body, so the detection of phosphorylated proteins is crucial for the study of various developmental disorders and human diseases. Our advanced antigen design tool OptimumAntigen™, proprietary immune adjuvants, optimized screening and purification technology of specific antibodies make our affinity purified phosphorylated specific antibodies highly sensitive in detecting the phosphorylation of highly complex protein mixtures. In addition to phosphorylated protein antibodies, GenScript also provides acetylation, methylation and other modified protein antibody preparation services.

Service Advantages

Advanced antigen design

High quality antibody

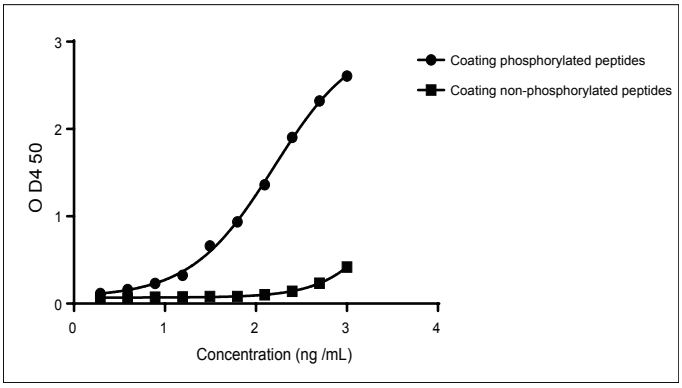
Short delivery cycle

Service Details

Service Content	Provided by customers	Deliverables	Cycle
Monoclonal antibody (5 Balb/c mice or 3 rats)	Target antigen/protein sequence	First delivery • Advantages of rabbit monoclonal antibody	First delivery 10-11 weeks
		Final delivery • 2-5 antibody hybridoma cell lines • 2 mg purified antibody • 2 mg phosphorylated peptide (<15 aa, 90% purity) • 2 mg non-phosphorylated peptide (<15 aa, 90% purity) • ELISA, MS, HPLC report	
		Commitment • 2-5 antibody hybridoma cell lines, at least one purified antibody • ELISA titer ≥1:256,000, cross-reaction with non-phosphorylated peptides < 10%	
Customized phosphorylated polyclonal antibody/monoclonal antibody service	Antigen provided by customers	Dependent on the content of the customized package	

Case 1:

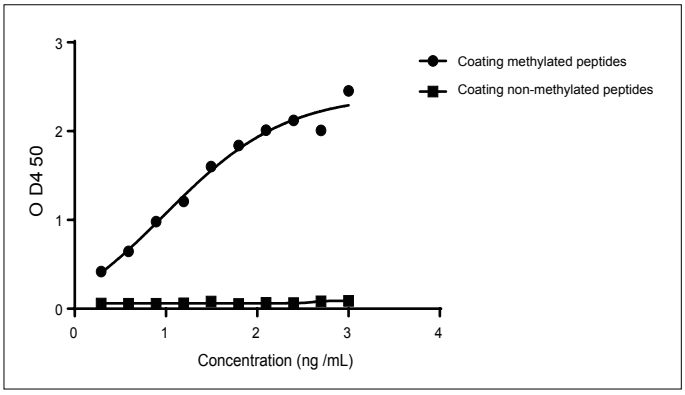
ELISA test of polyclonal antibody against phosphorylated peptide



The affinity of phosphorylated peptide (EC_{50}=157.5 ng/mL) was significantly increased compared with that of non-phosphorylated peptide.

Case 2:

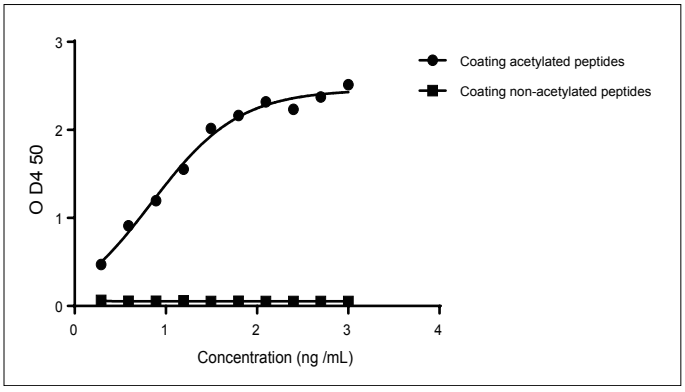
ELISA test of murine monoclonal antibody against to methylated peptide



The affinity of methylated peptide (EC_{50} = 9.6 ng/mL) was significantly increased compared with that of non-methylated peptide.

Case 3:

ELISA test of murine monoclonal antibody against to acetylated peptide



The affinity of acetylated peptide (EC_{50} = 6.9 ng/mL) was significantly increased compared with that of non-acetylated peptide.

Antibody Production

GenScript can provide large-scale antibody production services from microgram level to gram level for *in vitro* diagnosis and preclinical antibody drug discovery. In order to further meet the various needs of our customers, we can provide *in vivo* ascites production, *in vitro* shake-flask production, recombinant expression and other antibody production services, including mycoplasma detection and clone development with high-quality services. Certified by AAALAC and OLAW, GenScript ensures service quality at an international level.

Service Features

***In vitro* antibody production**

***In vivo* antibody production**

Flexible scale-up

Service Details

Antibody production	Ascites production	Roller bottle production	Recombinant production
Service features	• Used in vitro for poorly performing hybridoma cells in vitro culture (e.g. glycosylation, low yield)	• Scalable • Lot-to-lot consistency	• Scalable • High lot-to-lot stability • Sequence optimized • Highly customized
Volume	2-5 mL/mouse	>1 L	1mL-200 L

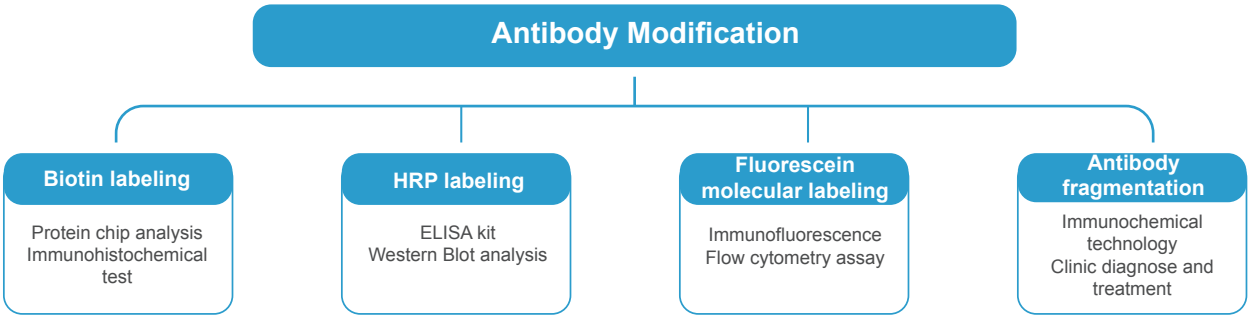
Antibody Modification

GenScript offers two kinds of modification services, antibody labeling and antibody fragmentation. The antibody labeling services mainly include biotin, HRP and fluorescein molecular labeling. Biotin labeling is a chemical reaction that covalently binds biotin to antibody molecules, while biotin labeled antibodies are mainly used in protein chip analysis and immunohistochemistry experiments. Horseradish peroxidase (HRP) is a commonly used enzyme in clinical and immunoassay, and HRP labeled antibody is widely used in ELISA kits and Western Blot analysis. Fluorescein isothiocyanate (FITC) is a widely used luciferin at present, and FITC labeled antibodies are commonly used in immunofluorescence and flow cytometry analysis. Labeled antibodies were used to perform qualitative, localized, or quantitative testing for the corresponding antigen in different immunoassays.

Service Details

Modification categories	Modification number
Biotin 、HRP 、FITC	Up to 10 mg
Alexa Fluor™ 488、Alexa Fluor™ 568、Alexa Fluor™ 647、APC、PE	Up to 5 mg

In order to meet the needs of more customers, GenScript also provides peptide antigen design, antiserum preparation, ELISA titer determination, Western blot detection and other antibody preparation related services. Equipped by our strong antibody service team, we provide customers with high quality antibody supporting services efficiently.



03

Resource Center

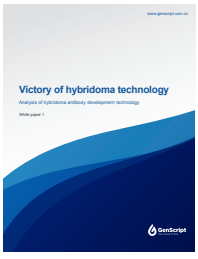
Technical Resources

White paper - GenScript's leading rabbit monoclonal antibody technology platform - MonoRab™

This white paper is a technical resource for scientists who are in the antibody engineering, therapeutics, and development areas of research and are working to optimize their system.

You will learn from this white paper:

- Advantages of rabbit monoclonal antibody
- Features of MonoRab™ platform
- Application of rabbit monoclonal antibody
- Cases

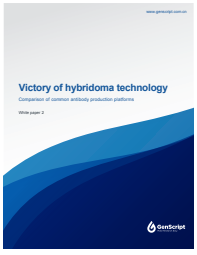


White paper 1 - Hybridoma Technology for the Win!

This manual is designed to give you a deeper understanding of the process and details of hybridoma.

You will learn from this handbook:

- Introduction of hybridoma technology
- Antigen design
- Host animal immunization
- Cell fusion produced hybridoma
- Hybridoma screening & selection
- Monoclonal antibody purification



White paper 2 - Hybridoma Technology for the Win!

This manual is a supplement to white paper 1, focusing on the advantages and characteristics of hybridoma technology.

You will learn from this handbook:

- Introduction of hybridoma technology
- Advantages of hybridoma technology in producing monoclonal antibodies
- Selection of hybridoma technology
- Misconceptions about hybridomas

Bioinformatics Tools

FoldArt™

A patented *in vitro* soluble and folding platform to recover purified proteins from inclusion bodies produced by high protein expression, including high hydrostatic pressure, small molecule additives and column folding technology to recover high-purity soluble proteins.

GenSmart™

GenSmart™ Codon Optimization technology is accessible and user-friendly. All the key parameters are integrated into the algorithm, which can be completed by filling in the basic information (such as sequence and host), and customized optimization of each gene leads to higher probability of obtaining functional and active proteins.

Solubility Tags

The *E.coli* expression vector with unique solubility marker developed by GenScript's professional scientists is specially used for the production of soluble recombinant protein.
The success rate of *E. Coli* project is greater than 95%.

OptimumAntigen™
Antigen Design Tool

The advantage of OptimumAntigen ™ is to avoid unexposed epitopes, and aim to have the required cross-reactivity to specific antigens with strong antigenicity, to identify the optimal coupling sites for the desired experiment, to apply the built-in peptide synthesis and solubility course, and to ensure antigen immune response.

FAQ

To help you solve the problem more quickly, you can scan the following QR code to enter the antibody resources page:

Questions	Answers
Q: How can you get high-quality antibodies?	A: We will discuss this in terms of antibodies based on your application needs. Antibodies need to have sufficient specificity and affinity. One kind of antibody may not meet all application needs, as different applications have different requirements for antibodies.
Q: What antigens are available for antibody development?	A: Antigens can be generally classified as follows: • Peptide • Fragment or full length of recombinant protein • Natural protein (purified) • Whole cell • DNA antigen • Micromolecule • Antibody (anti-idiotype antibody) • VLP virus-like particles
Q: What is the fusion ratio between myeloma cells and spleen cells during cell fusion?	A: It's usually 2:1.
Q: What animals can be used for polyclonal antibody service?	A: Rabbits, mice, rats, guinea pigs, goat, etc.
Q: What factors should be considered in peptide antigen design?	A: The antigenicity of peptides is greater than 0.6 (conventional); greater than 1.0 (rapid) Not difficult in peptide synthesis Homology with immunized animals: Less than 90% for mice, and less than 95% for rabbits Try to choose peptides with disorder value as antigens

Questions	Answers
Q: What is the difference between ascites preparation and roller bottle production of monoclonal antibodies?	A: The following aspects are included: 1. Ascites preparation BALB/C mice are used for ascites preparation to produce antibodies. Ascites are prepared from rat hybridoma cells in nude mice. Ascites have a short production cycle and a high yield, but the antibodies produced will contain 1%-10% of the mouse's own IgG. 2. Roller bottle production Antibodies are produced by *in vitro* culture. If there is a high requirement for experimental specificity and interference with the later application of rat IgG, it is suggested to choose roller bottle production. 2-5 mg antibody can be obtained from ascites of a mouse, and 15-20 mg antibody can be obtained from 1L roller bottle with SDS-PAGE purity above 90%.
Q: What antibody labeling services does GenScript offer?	A: HRP, FITC, Biotin, and other small molecule fluorescent dyes are available, and the customer is required to provide the name of the dye for our evaluation.
Q: How does GenScript use and store the antibodies we provide?	A: It is suggested to store the antibody separately after receiving it, and it can be stored at 4°C for 1 week; for long-term storage, it should be kept at -20°C or -80°C.
Q: What is special about the adjuvant that GenScript used?	A: GS adjuvant independently developed by GenScript has twice the immune effect than Freund's adjuvant.
Q: What are the advantages of GenScript rabbit monoclonal antibodies?	A: In the application of antibodies, affinity and specificity are the key points in evaluating the usefulness of antibodies. Rabbit monoclonal antibodies are a type of antibody with high specificity and affinity. KD of rabbit monoclonal antibody mostly ranges from 10^{-9} to 10^{-11}M, and some can reach 10^{-12}M. In addition, rabbit monoclonal antibody has higher diversity and is more likely to obtain target antibodies. With its unique advantages, the rabbit monoclonal antibody has been widely used in the field of life science, including basic research, diagnosis and treatment. GenScript provides high affinity, specificity and diversity of rabbit monoclonal antibody services, covering two R&D platforms (B cell cloning and single B cell sorting). Preparation of rabbit monoclonal antibody with B cell cloning of GenScript and 100% delivery of antigen binding positive clones can promote your R&D process at full speed!

Literature Published by Customers

Title: Prokaryotic viperins produce diverse antiviral moleculesProkaryotic viperins produce diverse antiviral molecules

Journal: *Nature* IF: 43.07 (2021-01)

Doi：10.1038/s41586-020-2762-2

Introduction: DNA synthesis and cloning into pBad/His A was performed by Genscript.

Title: Central memory CD8+ T cells derive from stem-like Tcf7hi effector cells in the absence of cytotoxic differentiation

Journal: *Immunity* IF: 22.845 (2020-11)

Doi：10.1016/j.immuni.2020.09.005

Introduction: Tcf7GFP mice were injected sub-cutaneously (s.c.) at the base of tail with a modified synthetic long Ovalbumin peptide (KKKKKLEQLEAAYSIINFEKL, termed KL-SLP) (15.86 nmole).(GenScript)

Title: Selenium-Doped Carbon Quantum Dots Act as Broad-Spectrum Antioxidants for Acute Kidney Injury Management

Journal: *Adv Sci (Weinh)* IF: 15.804 (2020-04-01)

Doi：10.1002/advs.202000420

Introduction: "Calnexin, Recombinant Calnexin, Rabbit anti-human, Mouse anti-human, Anti-Mouse IgG HRP.(GenScript etc)"

Title: Engineering Secretory Amyloids for Remote and Highly Selective Destruction of Metastatic Foci.

Journal: *Adv Mater* IF: 14.829 (2020)

Doi：10.1002/adma.201907348

Introduction: The amount of protein was quantified by WB using anti-His monoclonal antibody (Genscript).

Title: An ultra-stable cytoplasmic antibody engineered for in vivo applications.

Journal: *Nat Commun* IF: 12.124 (2020)

Doi：10.1038/s41467-019-13654-9

Introduction: HA tag (YPYDVPDYA)-fused scFv constructs (s3Flag-scFv-HA) were synthesised and codon-optimised for expression in mice using Genscript.

Title: Optogenetic regulation of endogenous proteins.

Journal: *Nat Commun* IF: 12.124 (2020)

Doi：10.1038/s41467-020-14460-4

Introduction: Anti-RAS monobody NS1, referred in this paper as “iB(RAS)” was synthetized by GenScript, using amino acid sequence from 5E95 Protein Data Bank entry for reverse translation53.

Literature Published by Customers

Title: The natural function of the malaria parasite's chloroquine resistance transporter

Journal: *Nat Commun* IF: 12.124 (2020-08)

Doi：10.1038/s41467-020-17781-6

Introduction: All of the host-derived peptides were custom-synthesised (GenScript; see Supplementary Data 1 and 2 for peptide sequences).

Title: A possible universal role for mRNA secondary structure in bacterial translation revealed using a synthetic operon

Journal: *Nature Communications* IF: 11.878 (2020-09)

Doi：10.1038/s41467-020-18577-4

Introduction: SDS gels [Genscript] and transferred to a PVDF membrane using an E-blot protein transfer apparatus (Genscript).

Title: Forces during cellular uptake of viruses and nanoparticles at the ventral side.

Journal: *Nature Communications* IF: 11.878 (2020)

Doi：10.1038/s41467-019-13877-w

Introduction: Cells were fixed with 4% (w/v) paraformaldehyde in PBS for 20 min Samples were washed twice in PBS and for membrane permeabilization cells were treated with 0.5% (v/v) Triton X-100 for 10 min Virusfactories were detected with an antibody against the non-structural reovirus μNS protein (anti μNS rabbit IgG, 1:200, generated by GenScript,) and secondary antibody Alexa Fluor 488 goat anti-rabbit IgG.

Title: Structure and dynamics of the active Gs-coupled human secretin receptor

Journal: *Nature Communications* IF: 11.878 (2020-08)

Doi：10.1038/s41467-020-17791-4

Introduction: This polyclonal antibody52 was raised by GenScript using the peptide antigen.

Title: On-demand drug release nanoplatform based on fluorinated aza-BODIPY for imaging-guided chemo-phototherapy

Journal: *Biomaterials* IF: 8.402 (2020-07-01)

Doi：10.1016/j.biomaterials.2020.120211

Introduction: Recombinant proteins were generated and obtained from GenScript.

Title: Impedimetric immunosensor for rapid and simultaneous detection of chagas and visceral leishmaniasis for point of care diagnosis

Journal: *Biosens Bioelectron* IF: 7.78 (2020)

Doi：10.1016/j.bios.2020.112573

Introduction: The T. cruzi-chimeric antigen IBMP 8.1 was obtained according to the methods reported by Santoset al. (2016a). Briefly, the T. cruzi recombinant gene was optimized for Escherichia coli expression and synthesized by a commercial supplier (GenScript).

Literature Published by Customers

Title: Soluble collectin-12 mediates C3-independent docking of properdin that activates the alternative pathway of complement

Journal: *Elife* IF: 7.725 (2020)

Doi：10.7554/eLife.60908

Introduction: DNA encoding human properdin was synthesized (GenScript) with the endogenous signaling peptide and a C-terminal 6xHis-tag and cloned into the pCEP4 mammalian expression vector using HindIII and BamHI restriction sites.

Title: Berberine Chloride is an Alphavirus Inhibitor That Targets Nucleocapsid Assembly

Journal: *MBio* IF: 6.956 (2020-06)

Doi：10.1128/mBio.01382-20

Introduction: ".Protein purity was confirmed by SDS-PAGE followed by Coomassie staining and Western blotting (WB) analysis using polyclonal antibody (PAb) to the Strep tag (GenScript)."

Title: Risk1, a Phosphatidylinositol 3-Kinase Effector, Promotes Rickettsia typhi Intracellular Survival

Journal: *MBio* IF: 6.956 (2020-06)

Doi：10.1128/mBio.00820-20

Introduction: Codon-optimized recombinant proteins for wild-type (WT) full-length Risk1 (Risk1 WT), and the catalytically dead mutant (Risk1 H297A) were expressed and purified by GenScript.

Title: A CD63 Homolog Specially Recruited to the Fungi-Contained Phagosomes Is Involved in the Cellular Immune Response of Oyster

Journal: *Front Immunol* IF: 6.429 (2020)

Doi：10.3389/fimmu.2020.01379

Introduction: After incubating at 18°C for 2 h followed by three times of washing, 100 μl mouse anti-His tag monoclonal antibody (Genscript) diluted to 1:2,000 was added and incubated at 37°C for 1 h.

Title: Calreticulin S-Domain Binds to Human Complement C1q to Interfere With C1q-Mediated Immune Functions

Journal: *Front Immunol* IF: 6.429 (2020-11)

Doi：10.3389/fimmu.2020.572326

Introduction: Endotoxin removal was confirmed using the ToxinSensor Endotoxin Detection System (GenScript).

Title: Properdin Is a Key Player in Lysis of Red Blood Cells and Complement Activation on Endothelial Cells in Hemolytic Anemias Caused by Complement Dysregulation

Journal: *Front Immunol* IF: 6.429 (2020)

Doi：10.3389/fimmu.2020.01460

Introduction: Anti-properdin hybridoma cell lines were developed by Genscript usingour purified human properdin.

Literature Published by Customers

Title: Receptor-Bound Perfluoroalkyl Carboxylic Acids Dictate Their Activity on Human and Mouse Peroxisome Proliferator-Activated Receptor γ

Journal: *Environ Sci Technol* IF: 6.198 (2020-07-01)

Doi：10.1021/acs.est.0c02386

Introduction: Human and mouse PPARγ 138 ligand binding domains (LBDs) were prepared by Genscript.

Title: Extracellular Histones Inhibit Fibrinolysis through Noncovalent and Covalent Interactions with Fibrin

Journal: *Thromb Haemost* IF: 6.094 (2020-11)

DOi: 10.1055/s-0040-1718760

Introduction: Anti-his tag anti- bodies (A00186) were from Genscript .

In terms of services and products, GenScript have been cited nearly 10,000 times by more than 1300 journals of biomedicine such as *Cell, Nature, Science and PNAS*.

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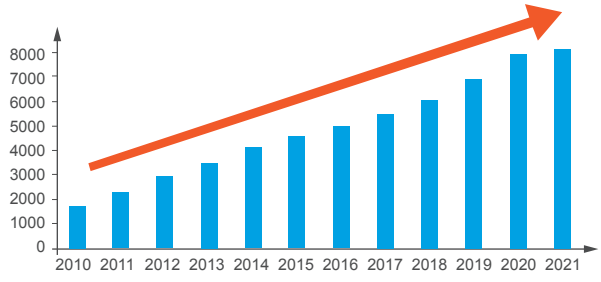

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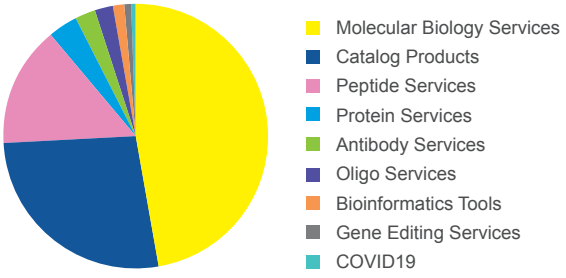

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04

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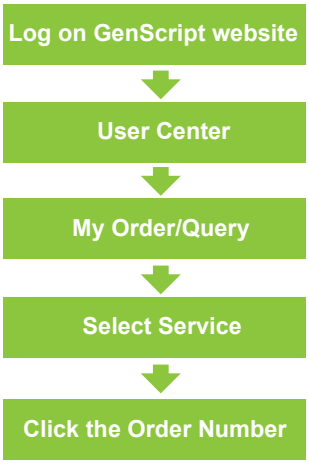

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