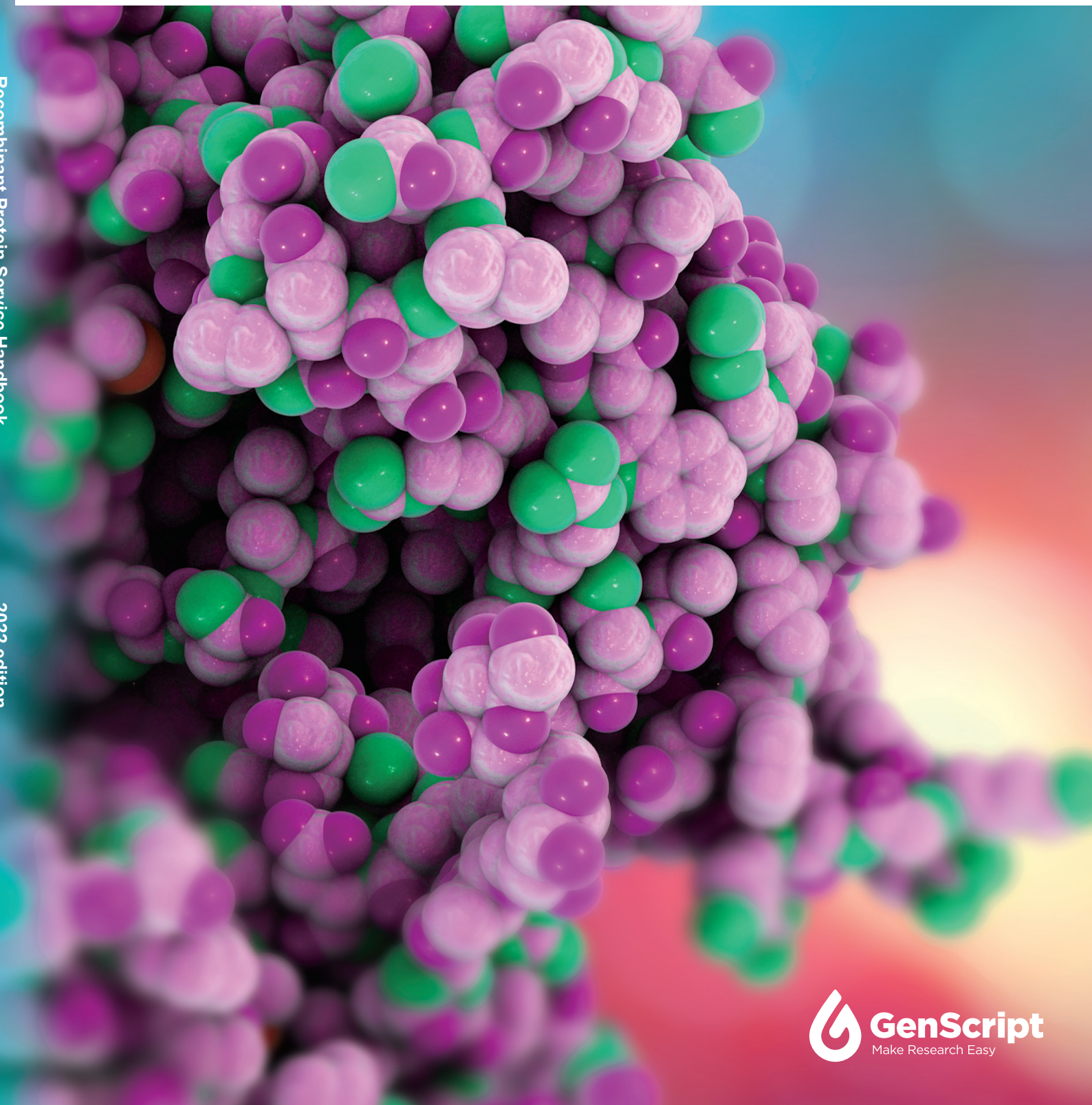
PROTEIN

Recombinant Protein Service Handbook

www.genscript.com
[2022 edition]



GenScript
Make Research Easy

Recombinant Protein Service Handbook
2022 edition


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 **5200 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

CONTENTS

01

Recombinant Protein Expression Platform

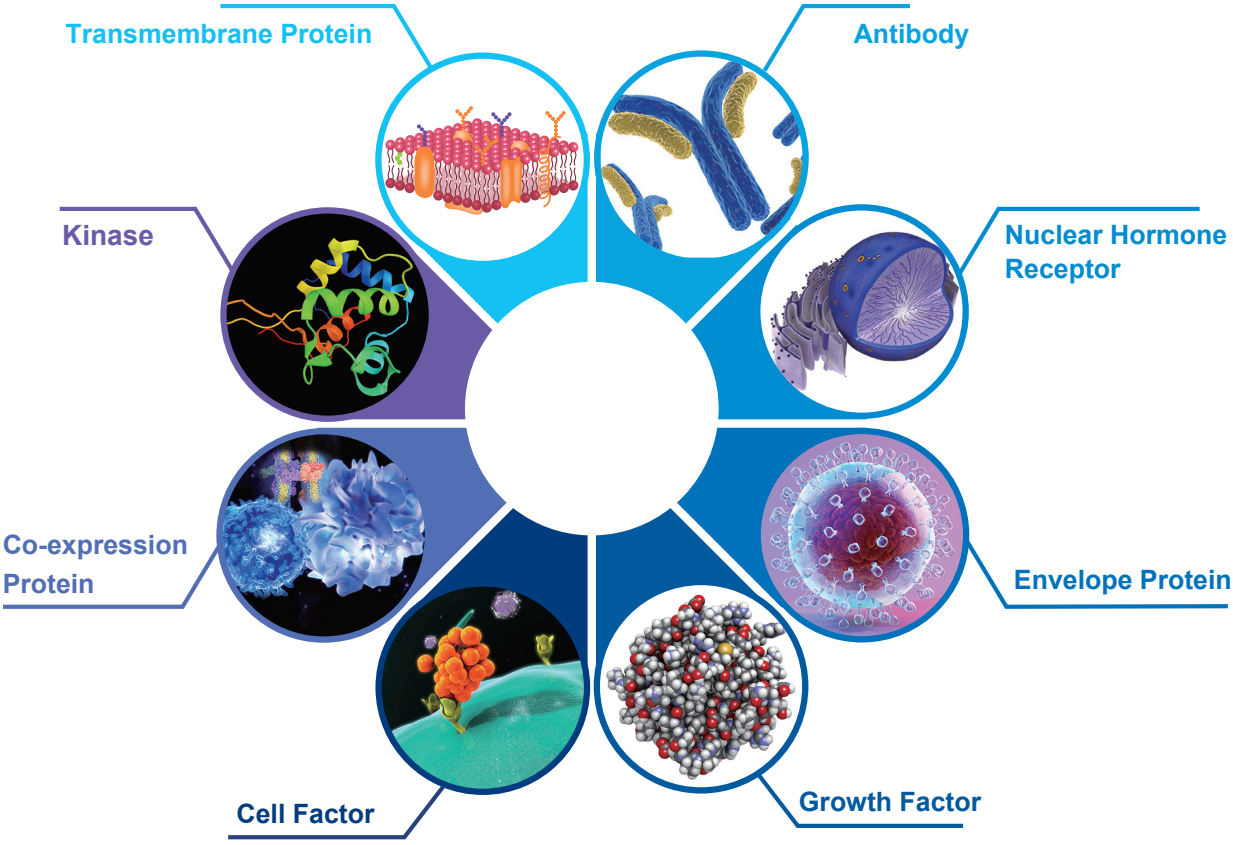
Protein Production Capacity

Successful delivery of **>40,000** batches of high-quality protein

Successful delivery of **>30,000** batches of recombinant antibody

Success rate **>98%**

Successful delivery of multiple proteins



Application Range

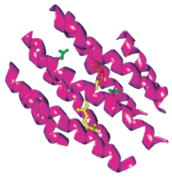
GenScript has over 18 years of experience in protein expression and purification. The Company combines an experienced production team with advanced equipment to ensure that it can meet the different needs from a variety of customers, including complex projects such as protein expression in multiple systems followed by purification and specific analysis.

- ✓ Target Identification and Verification
- ✓ Enzyme Identification
- ✓ High Throughput Screening
- ✓ Antibody Preparation
- ✓ Structural Biology Research
- ✓ Eukaryotic Protein Analysis
- ✓ Isotope Labeled Protein Tag Research
- ✓ SAR (Structure-Acitivity Relationships)

Workflow

Submit gene sequence by email

Gene synthesis and subcloning



Protein expression and purification

QC by multiple methods

Delivery of expressed protein samples

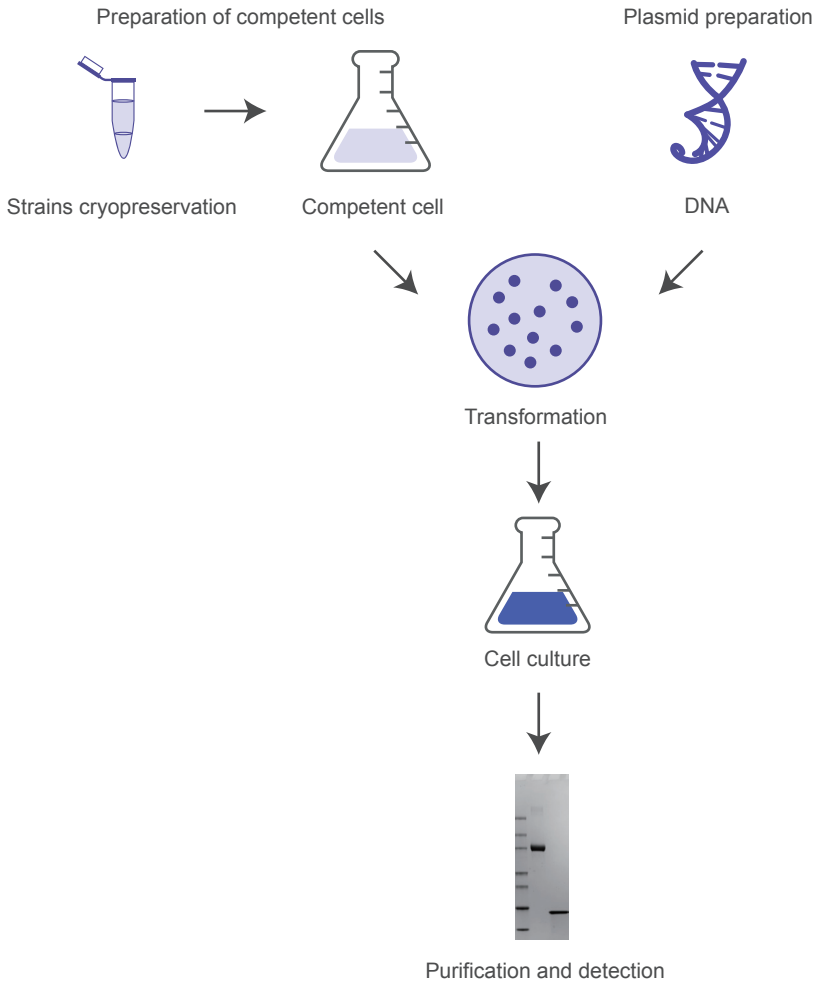
Prokaryotic Protein Expression System

The prokaryotic protein expression system is both commonly used and economical. Represented by the *E. coli* expression system, it has the advantages of clear genetic background, low cost, high expression and relatively simple separation and purification of expressed products.

Service Type

- BacPower™ customized protein expression service
- Bacterial fermentation service

Service Process



Prokaryotic Protein Expression System

BacPower™ customized protein expression service

Customized services are available for proteins with special experimental needs such as structural analysis and functional analysis, and customers can provide experimental protocols and intermediate samples. GenScript offers customers with flexible services to meet different needs: from codon optimization to gene synthesis, to small-scale testing and amplification, as well as protein purification and property analysis.

Service Advantages

Flexible

Fast cycle

Multiple customization options

Service Content

Milestones	Specifications	Deliverables	Timeline*
① Gene synthesis, Cloning, Plasmid Prep	• Gene synthesis and Codon optimization • Subcloning into appropriate expression vector • Plasmid Prep and DNA QC	• Optimized gene sequence report • Protein in buffered solution with your specified amount & purity • QC data	≤2 weeks
② Protein Expression Evaluation	• Transform plasmids into appropriate bacterial expression strain • Protein expression evaluation and optimization		≤1 week
③ Protein Purification, Tag Removal (Optional) & Refolding (Optional)	• Scale up protein expression using optimized conditions • Purification to reach desired protein amounts and purity • Tag removal and separation of tag-free protein (if requested) • Refolding in case of insoluble protein (performed only if required)		≤1 week
④ QC & Delivery	• Purity analysis and Detection using SDS-PAGE and Western Blot (for tagged protein) • Bradford assay for quantitation • Protein delivery		≤1 week

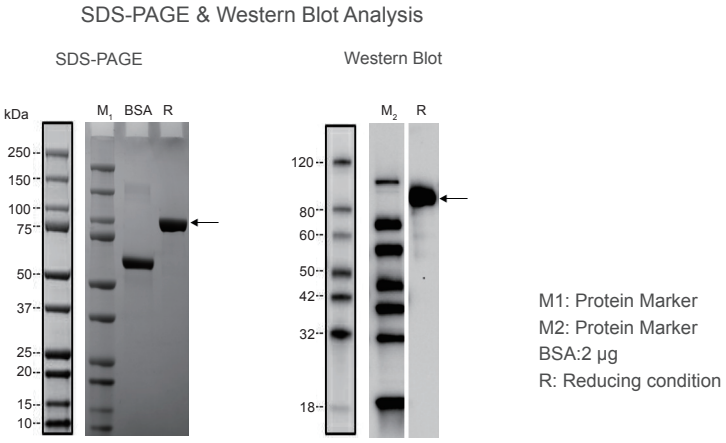
Note:

*All timelines are approximate. In most cases, we deliver within 4-6 weeks. However, optional steps might prolong the overall timeline slightly.

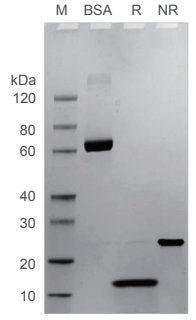
For any large scale protein production requirements, you could try our fully customizable E. coli fermentation services. Talk to our technical account managers for more details.

Cases

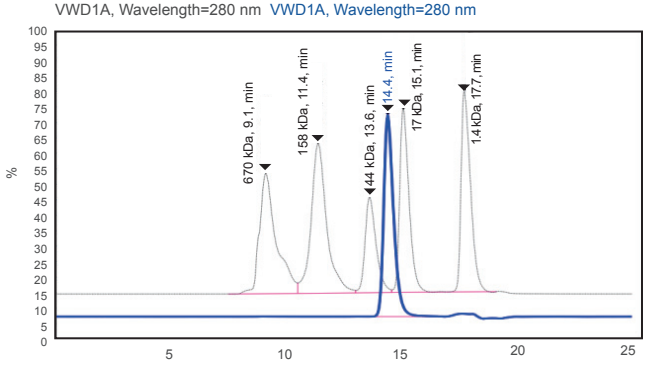
◆ Case 1: Protein expression

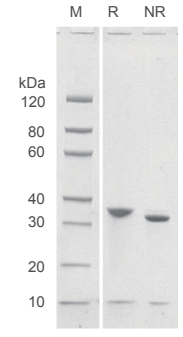


◆ Case 2: Professional refolding service

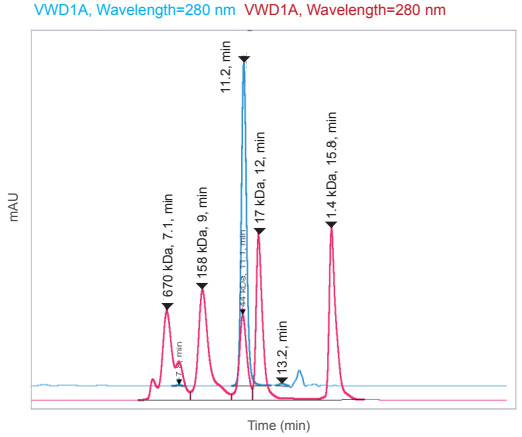


Human BMP9 12.2 kDa
BSA and target protein: 2 μg





Human MHC I HLA 34.7 kDa



Bacterial fermentation service

High purity protein is a prerequisite for scientific research such as high-throughput screening, functional analysis, and structural biology. However, large-scale protein production and purification is not only a bottleneck for some scientists, but also requires some core equipments.

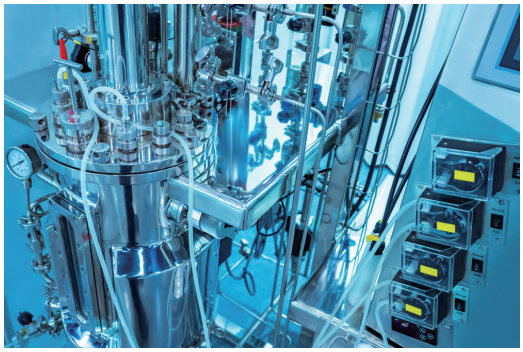
GenScript provides industrial scale bacterial fermentation services to meet customers' needs for specific projects, with fermentation capacity up to 2,000 L.

Service Advantages

- Flexible fermentation capacity of 10-2,000 L
- Multiple large-scale protein purification methods
- Gene-to-protein integrated services
- Cost-effective

Service Content

GenScript bacterial fermentation services are tailored to the needs of customers, from optimization of protein expression, microbial culture conditions, and scale-up cultures, to large-scale production, protein purification, and identification. The fermentation capacity is up to 2000 L. GenScript can provide you with up to 3 tons of transformed bacterial cytoplasm (cell pellet) or gram level recombinant proteins with purity up to 98%.



Delivery of Products

- Bacterial cytoplasm (cell pellet)
- Batch record
- QC report

Baculovirus-insect Cell Expression System

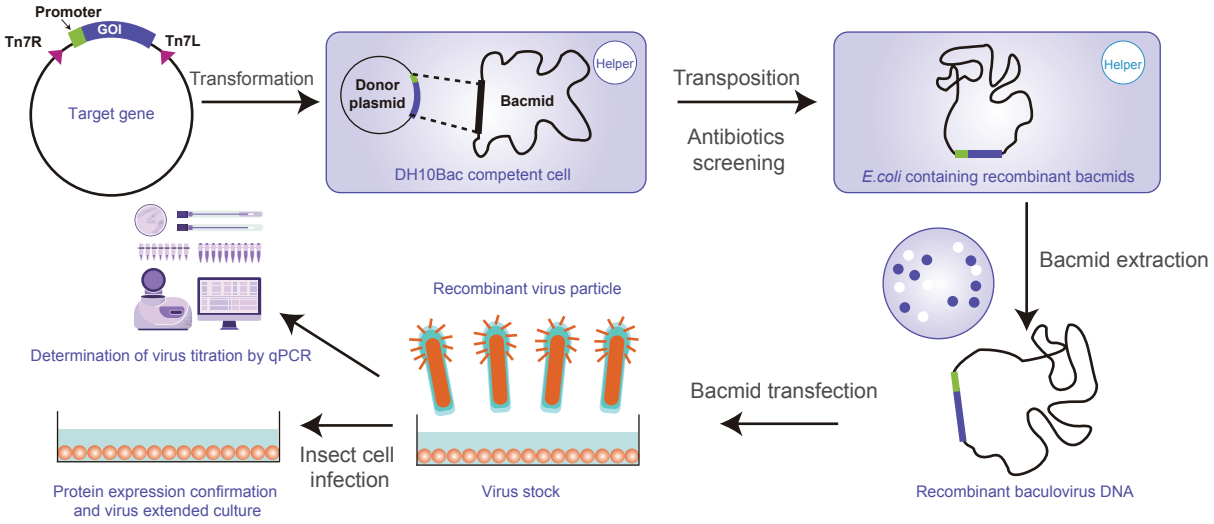
The baculovirus-insect cell expression system is a powerful and commonly used system, which is suitable for the preparation of large-scale recombinant proteins. It is considered as a potent tool for protein expression because of its simple process, high expression level, and post-translation modifications.

Covering virus preparation and large-scale protein production, GenScript baculovirus protein expression system provides corresponding services for pharmaceutical companies, biotechnology companies, and academic research institutions. Based on the codon preference of Sf9, Sf21, Hi5 and other insect cell lines, GenScript provides free codon optimization for you to effectively improve protein expression.

Service Advantages

- Success rate for protein expression above 90%
- Multiple expressing cell (Sf9, Sf21, Hi5 and others)
- Fast delivery of plasmid to protein
- One-stop service from gene to protein

Service Workflow



Baculovirus-insect Cell Expression System

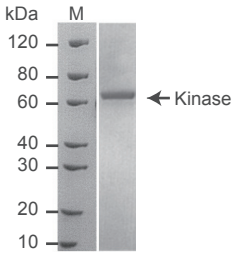
Milestones	Specifications	Delivery	Cycle
① Gene synthesis and subcloning	• Subcloning into one or more expression vectors	Construct(s) report	2 weeks
② Virus generation	• Generation of recombinant Bacmid DNA • Transfection of insect cell with recombinant Bacmid DNA • Generation of P1 stock (Low titer), P2 stock (High titer), and determination of virus titer by Quantitative-PCR • Verify the protein expression for P1 and P2 generation by western blot with anti-His/GST or other antibody	5 ml, >10^7 pfu/ml virus stock	2~3 weeks
③ Pilot expression evaluation	• Infection of insect cells with P2 stock • One step affinity purification • SDS-PAGE gel and Western blot	Expression data	1.5 weeks
④ 1 L expression and purification	• 1 L insect cell expression and purification • One-step purification • QC by SDS-PAGE, Western blot[2], and LC-MS/MS coverage[2]	Purified proteins QC data	1~2 weeks

Note:

1. Listed is the typical turnaround time at GenScript. Please note that additional requirements may incur additional steps, and likewise longer processing time.
2. Other QC data, such as LC-MS/MS coverage, N-terminal sequencing and LC-MASS are available with additional charge.

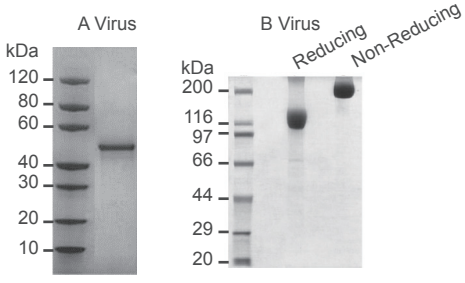
Cases

◆ Case 1: Kinase protein expression



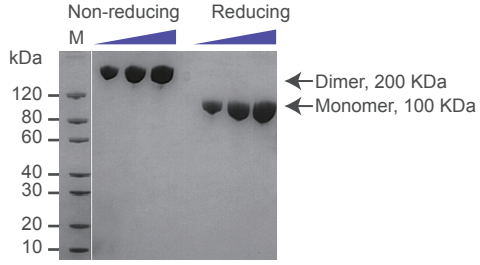
Sf9 cells expressed kinase with 95% purity and yield to 10 mg/L.

◆ Case 2: Virus protein expression



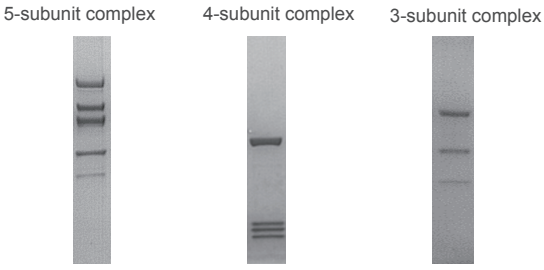
The virus protein was successfully expressed in the insect baculovirus system.

◆ Case 3: Macromolecule weight protein expression



Hi5 cells expressed macromolecule weight protein (100 kDa) with 95% purity and yield to 10 mg/L.

◆ Case 4: Multisubunit complex protein



The multisubunit complex protein was successfully expressed in the insect baculovirus system.

Mammalian Cell Expression System

The mammalian cell expression system has the process of both post-translational modifications and functional folding during the protein expression. This feature allows the expressed protein closer to the natural state of mammalian proteins in terms of molecular structure, physical and chemical properties and biological function, with the necessary spatial structure and modification, and resulting in the specific biological activity. It also makes the mammalian cell expression system more widely used in the development and production of functional proteins, antibodies and clinical vaccines.

Service Type

- TurboCHO™ high throughput expression (TurboCHO™ HT)
- Expression of gram level protein (TurboCHO™ Express, TurboCHO™ HP)

Expression service of high-throughput antibody (CHO-HT)

High throughput expression is a cost-effective option for producing small-scale recombinant antibodies and proteins. GenScript HTP service is a one-stop service from gene synthesis to purified antibody/protein, and we look forward to assisting your research at full speed!

From gene synthesis to protein or antibody, fast delivery takes only 2-week, and triple QC testing can ensure the delivery quality! The whole process is highly automated, the samples are monitored by the respective QR codes, and the whole process can be traced. Coordinating with the Biosecurity Law, GenScript can provide you with comprehensive support including materials in a more standardized and comprehensive manner for your downstream R&D projects.

Service Advantages

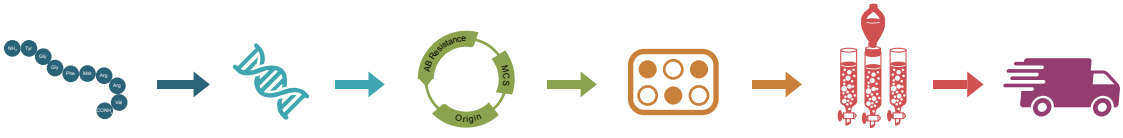
Fast delivery in 2-week

Traceability of the whole process

Comprehensive improvement of the delivery

Multi-step QC

Service Process



Antibody sequences provided by customers

Codon optimization for sequence analysis

High throughput gene synthesis

High throughput antibody expression

Purification and QC testing

Delivery your recombinant proteins

Service Content

Volume	1 mL	4 mL	20 mL
Timeline	Fast delivery in 2-3 week		
Cell line	CHO		
Minimum order quantity	8		
Protein types	Standard antibody, antibody fragment (customized recombinant protein), bispecific antibody		
QC*	SDS-PAGE, endotoxin control and sampling inspection (other customized QC items)		
Process	Gene synthesis to antibodies (customized recombinant protein)		
Purification	One step purification (customized multi-step purification)		
Additional services	Multiple ELISA, mass spectrometry, affinity determination, phenotypic mapping, cross reaction, in vivo/in vitro experiments		

- QC*: LC-MS, ELISA analysis, quantitative detection of endotoxin, peptide mapping and other QC methods;
- Antibody type: chimeric antibody/Fab/scFv/bispecific antibody/class switching antibody/nanobody, etc;
- Downstream expansion experiment: affinity experiment, cross-reaction experiment, in vitro and in vivo efficacy, epitope analysis, stability testing, sterility testing, etc.

Expression of gram level protein (TurboCHO™ Express, TurboCHO™ HP)

GenScript developed a new generation of proprietary CHO transient expression that significantly increased yields and reduced production time. CHO transient expression is a cost-effective protein expression scheme that enables small-scale (microgram) to kilogram-scale protein production with good consistency in downstream applications.

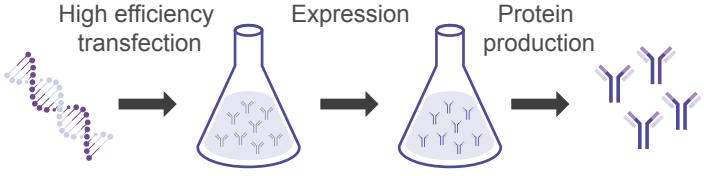
Service Advantages

High expression titer

Validated consistency

Easy to scale up

Service Process

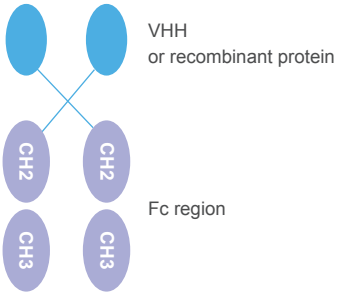


Service Content

	CHO-Express	CHO-HP
Demanded quantity of protein	mg～g	g～kg
Expression volume	40 mL～200 mL	200 mL～200 L
Timeline	3 weeks	6 weeks
Deliverables	• Purity: ≥ 95% (antibody) • Endotoxin: ≤ 1 EU/mg (antibody)	
Default QC testing	SDS-PAGE, SEC-HPLC, endotoxin, A280, LC-MS (only for antibody)	
Additional QC testing	Affinity experiment, Cross reactivity experiment, epitope classification, *in vitro* and in vivo efficacy, epitope analysis, sterility testing, etc.	
Purification	Multi-step purification available	

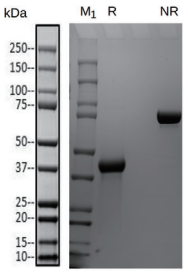
Cases

◆ **Case 1:** Fc fusion protein expression

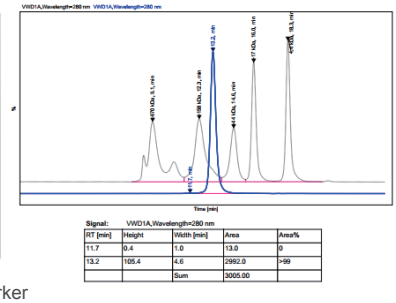


Molecule 1: VHH + Fc

SDS-PAGE



SEC-HPLC

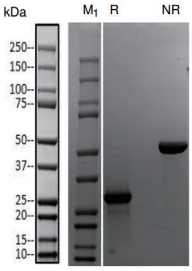


RT [min]	Height	Width [min]	Area	Area%
11.7	0.4	1.0	13.0	0
13.2	105.4	4.6	2992.0	>99
		Sum	3005.00	

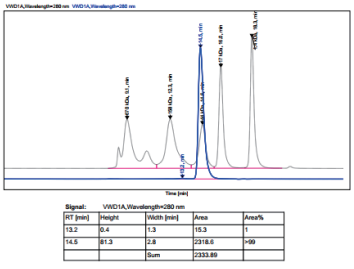
Lane M1: Protein Marker
R: Reducing condition
NR: Non-reducing condition

Molecule 2: Recombinant protein + Fc

SDS-PAGE



SEC-HPLC

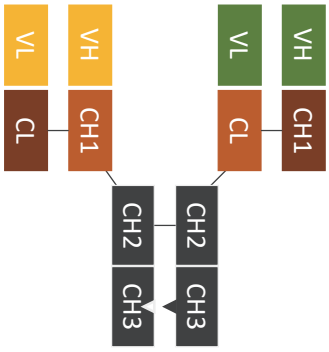


RT [min]	Height	Width [min]	Area	Area%
13.2	0.4	1.3	15.3	1
14.5	81.3	2.8	2318.6	>99
		Sum	2333.89	

Lane M1: Protein Marker
R: Reducing condition
NR: Non-reducing condition

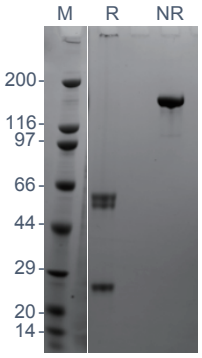
Product purity > 99% by SEC-HPLC

◆ **Case 2:** Expression of asymmetric bispecific antibody

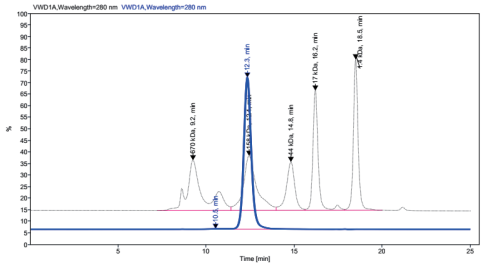


CrossMab (KiH backbone)

SDS-PAGE



SEC-HPLC



Signal:	VWD1A,Wavelength=280 nm			
RT [min]	Height	Width [min]	Area	Area%
10.5	0.3	1.6	16.4	0
12.3	144.7	5.2	4326.1	>99
		Sum	4342.53	

The expression of assymmetric bispecific antibody was 299 mg/L, and the purity was 99% by SEC-HPLC

Contact US and send email to protein@genscript.com

02

Cell Line Development Platform

Lentivirus Packaging Service

Lentivirus, a type of retrovirus, has become an essential tool for gene transduction. Lentiviruses can introduce target genes into cells that are difficult to transfect, such as primary cells and stem cells, thus greatly improving the transfection efficiency of target genes. Lentiviruses are also widely used in the construction of stable cell lines.

GenScript introduces lentivirus packaging services with diverse titers and specifications, flexible options and fast delivery (as fast as 2 weeks).

Service Advantages

Cost-effective

Less cost for the same amount of virus

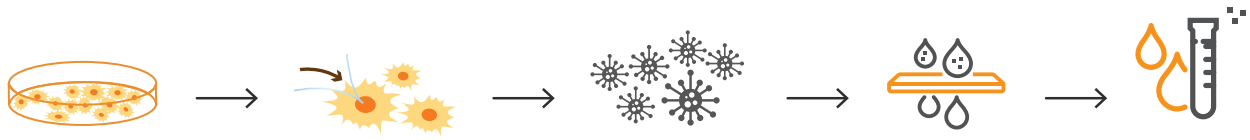
Fast delivery

As fast as 2 weeks

Strict quality control

Stringent testing standards

Service Process



Cell culture → Transfection → Virus collection → Purification → Titer testing

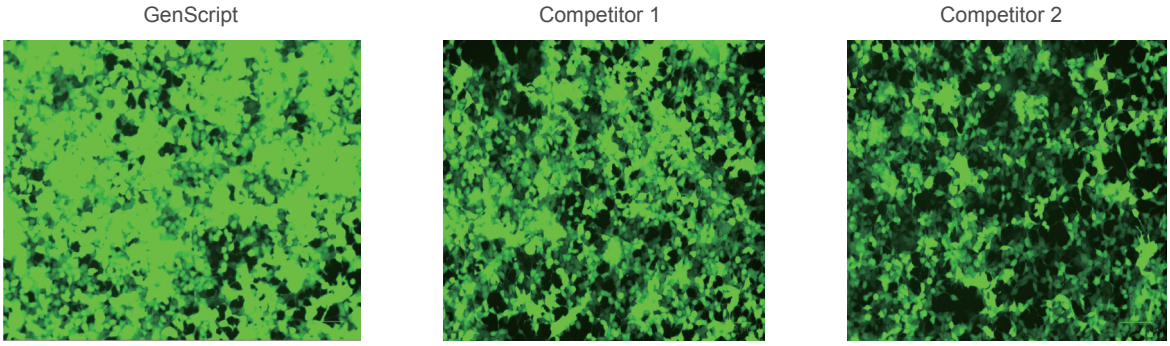
Service Content

Service	Titer	Specification	QC standard	Cycle
Lentivirus packaging (SC1394-VP)	>10^7 IFU/mL	1 mL	p24 ELISA Mycoplasma test	2~3 weeks
	>10^7 IFU/mL	2 mL		
	>10^8 IFU/mL	1 mL		
	>10^8 IFU/mL	2 mL		
	>10^9 IFU/mL	0.1 mL		
	>10^9 IFU/mL	0.2 mL		
	Customized services			

For customized services, please send an email to protein@genscript.com

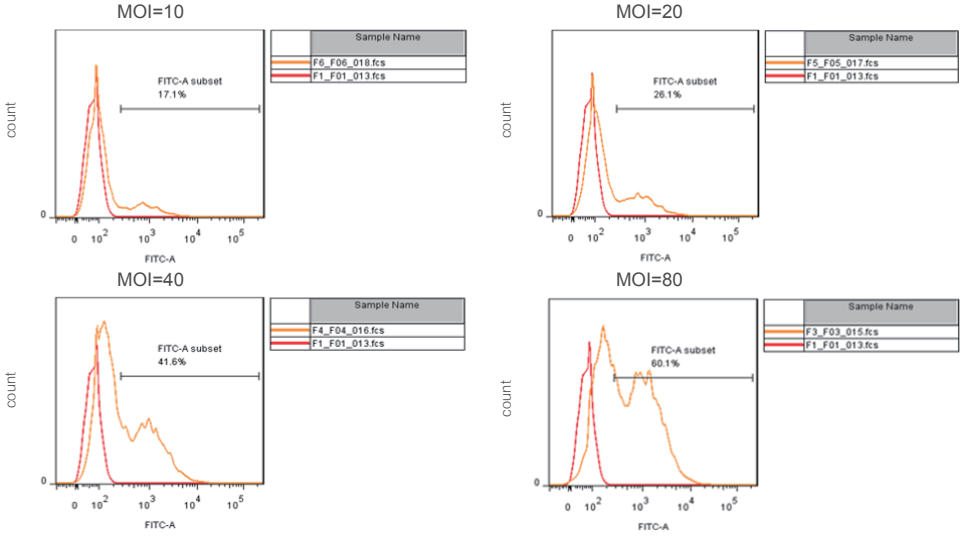
Cases

◆ Case 1: Lentivirus transductional performance



EGFP lentivirus was packaged by GenScript, Competitor 1 and competitor 2, respectively. After the determination of titers, they were adjusted to the same MOI to infect the same amount of 293T cells. Fluorescent photographs were taken 72 hours after infection. GenScript lentivirus has the highest transductional efficiency.

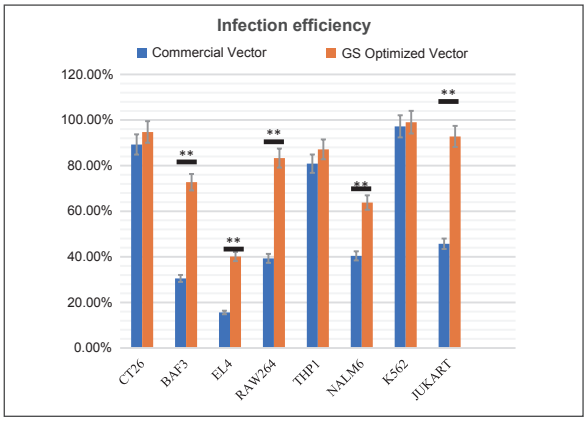
◆ Case 2: GFP lentivirus infection Jurkat MOI testing



MOI	GFP	Infection time
10	17.1%	72 h
20	26.1%	72 h
40	41.6%	72 h
80	60.1%	72 h

Jurkat cell is a type of T lymphocyte that is difficult to transfect. Jurkat cells were infected with GenScript third-generation GFP lentivirus packaging plasmid system and tested to achieve an infection rate of 60.1% at MOI=80.

◆ Case 3: Optimized vector performance



Cell line	Name	MOI
CT26	Mouse colony	100
BAF3	Mouse B cell	
EL4	Mouse T lymphoma	
RAW 264.7	Monocyte/macrophage-like cells	
Jurkat	Human acute T cell leukemia	
K562	myelogenous leukemia cell line	
NALM6	B cell precursor leukemia cell	
THP-1	Acute monocytic leukemia	

MOI: Multiplicity of infection

Our optimized vector can help you to achieve higher infection rate even in hard-to-transduce cell line.

◆ Case 4: GenScript lentivirus packaging system with different target gene length testing

Gene name	Target gene length	Titer (×10^8 IFU/mL)
Gene 1	3.8 kb	3.43
Gene 2	4.7 kb	3.58
Gene 3	5.5 kb	1.84
Gene 4	6.6 kb	2.61

In general, when the length of the target gene exceeds 3000 bps, it is difficult to reach a high titer level, and even can not be packaged. GenScript's lentivirus packaging system can reach up to 6.6 kb. GenScript can guarantee a titer of 10^8 IFU/mL for conventional genes (moderate GC content, no complex repeats, no cytotoxic gene and less than 5 kb).

Downstream Application

- Stable cell line construction, such as overexpression cell line, functional assay cell line and knockdown cell line.
- Construction of gRNA virus library.
- Improve the efficiency of gene transduction in diffficult-to-transfect cell lines, such as primary cells, iPS cells, other stem cells and non-dividing cells.
- Provide high-quality lentivirus for animal model experiments.
- Clinical treatment research, such as gene therapy, CAR-T cell construction.
- Vaccine research.

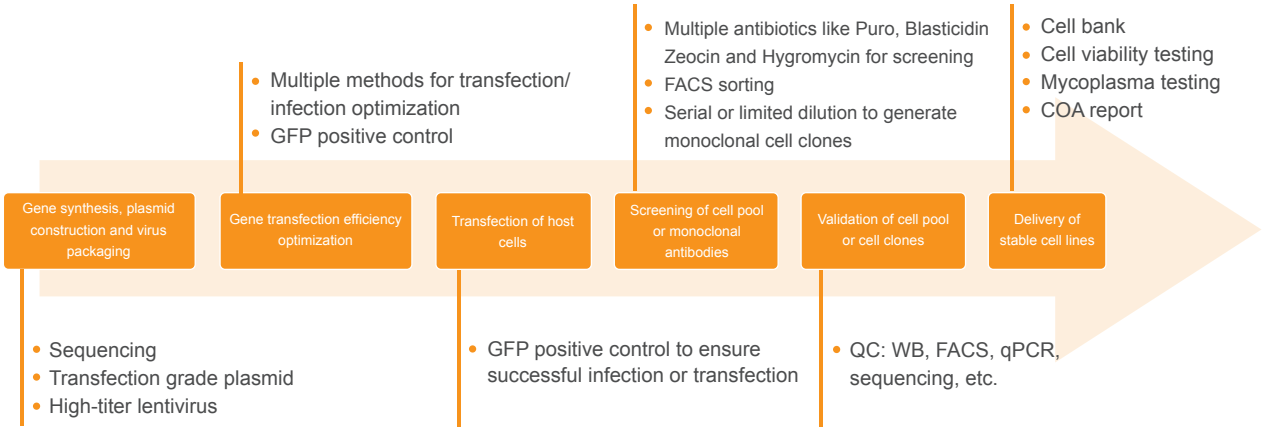
CellPower™ cell line development service

Stable cell lines are widely used in drug discovery, toxicity testing and basic research. Long-term stable expression of a gene of interest (GOI) is usually achieved by plasmid transfection or virus transduction which containing the expression cassette of GOI together with a selection marker (antibiotics or fluorescent proteins). Recombinant stable cell line development at GenScript includes constitutive expression and inducible expression. In addition, we developed lentivirus based CRISPR/Cas (Lenti-CRISPR/Cas9) technologies and platforms, in order to meet the increasing need of GOI-knockout cell lines, especially for difficult-to-transfect cells.

Service Advantages

- Multiple transfection or infection modes
- 250+ target cells experience
- Extensive protein expression experience
- 180 species of mycoplasma testing
- Multiple detection methods

Service Process



Service Content

Service number	Delivery	Cycle
CellPower™ Constitutive overexpression cell line	• Report of cell line testing • 2 cryovials of pooled cells with 10^6 cells/vial • Report of cell clone generation • Report of cell clone screening • 2 cryovials of single clone with 10^6 cells/vial • COA report	• Stable pool: Starting from 8 weeks • Single clone: Starting from 12 weeks
CellPower™ Reporter cell line	• Report of cell line testing • 2 cryovials of pooled cells with 1×10^6 cells/vial • Report of cell clone generation • Report of cell clone screening • 1 cryovials of single clone with 10^6 cells/vial • COA report/analytical method	• Stable pool: Starting from 10 weeks • Single clone: Starting from 14 weeks
CellPower™ Inducible overexpression cell line	• Report of cell line testing • 2 cryovials of pooled cells with 1×10^6 cells/vial • Report of cell clone generation • Report of cell clone screening • 1 cryovials of single clone with 10^6 cells/vial • COA report	• Stable pool: Starting from 10 weeks • Single clone: Starting from 15 weeks

In addition to the above lentivirus cell line development services, GenScript also offers CRISPR/Cas9 cell line development services.

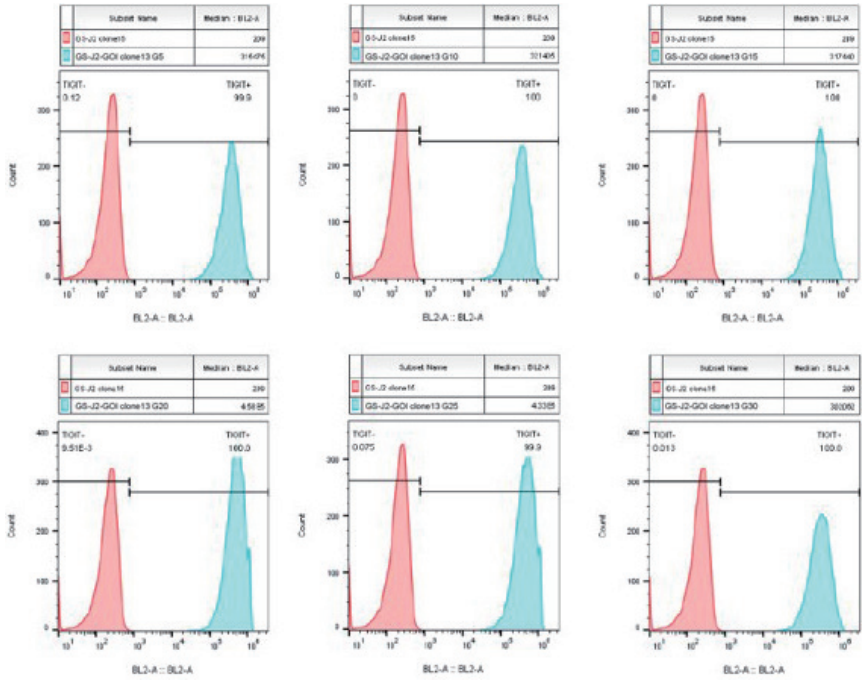
CRISPR is a powerful tool for research and discovery of drugs that change multiple genes in cells in highly targeted ways. Compared with other gene editing forms, such as TALENs and zinc finger nuclease (ZFN), CRISPR/Cas9 has the advantage of being easier to manipulate and more efficient in performing biallelic modifications.

GenScript provides GenCRISPR™ based gene editing services that allow the development of gene-edited cells for multiple genes using multiple mammalian cell lines.

For more information on CRISPR-related services, please refer to the GenScript Gene-editing Services Manual.

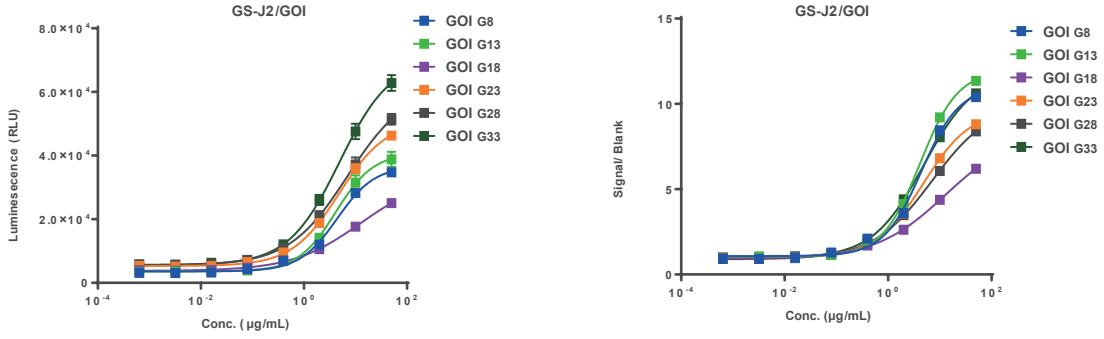
Cases

◆ **Case 1:** Stability testing of reporter gene overexpression



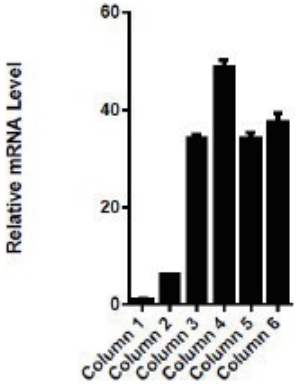
Expression stability test in 30 generation

Reporter gene testing



Reporter gene expression stability test in 33 generation

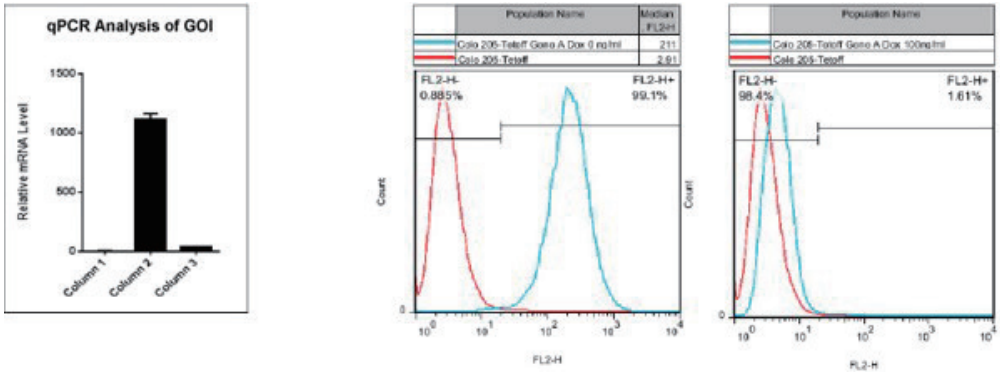
◆ **Case 2:** Tet on inducible assay cell line



Verification of Gene A mRNA expression by qPCR
Column 1. Hep-G2 parent cell
Column 2. Hep-G2/Tet on/Gene A Dox 0 ng/mL
Column 3. Hep-G2/Tet on/Gene A Dox 500 ng/mL
Column 4. Hep-G2/Tet on/Gene A Dox 1000 ng/mL
Column 5. Hep-G2/Tet on/Gene A Dox 1500 ng/mL
Column 6. Hep-G2/Tet on/Gene A Dox 2000 ng/mL

Verification of Gene A protein expression by Western Blot
Column 1. Hep-G2 parent cell
Column 2. Hep-G2/Tet on/Gene A Dox 0 ng/mL
Column 3. Hep-G2/Tet on/Gene A Dox 1000 ng/mL

◆ **Case 3:** Tet off inducible assay cell line



Verification of Gene A mRNA expression by qPCR and FACS
Column 1: Colo 205/Tet off
Column 2: Colo 205/Tet off_Gene A 0 ng/mL Doxycycline
Column 3: Colo 205/Tet off_Gene A 100 ng/mL Doxycycline

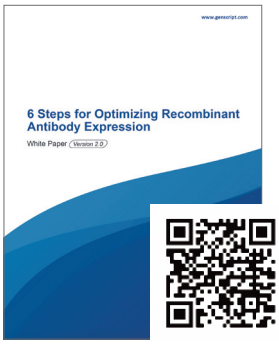
Downstream Application

- Drug screening.
- Gene expression regulation.
- Functional assay.
- FACS screening of membrane protein antibody.
- Localization studies or live imaging with fluorescence labeled proteins.
- *In vivo* tumor immunity research.

03

Resource Center

Technical Resources



White Paper - 6 Steps to Optimize Your Recombinant Antibody Expression

This white paper provides technical resources for scientists in the fields of antibody engineering, therapeutics and research and development, and for optimizing expression systems.

You will learn from this white paper:

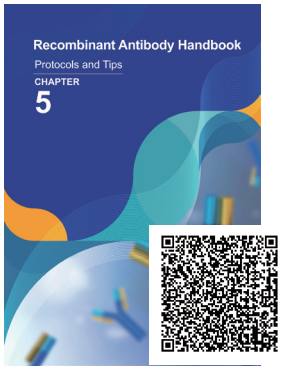
- Choosing an expression host system
- Designing an expression vector
- Optimizing the cell expression system
- Choosing between transient expression and stable expression
- Choosing a suitable transfection method
- Choosing a suitable purification method

4 Strategies for Boosting Transient Protein Expression

This application note is a free educational and technical resource for biomedical scientists studying proteins or antibodies.

You will learn from this handbook:

- Designing an expression vector
- Utilizing a fusion partner
- Increasing protein stability
- Improving protein purification



Recombinant Antibody Handbook - Processes and Strategies

This handbook is designed to help you improve the purity and yield of recombinant antibodies.

You will learn from this handbook:

- Recombinant expression and purification of IgM and a bispecific antibody
- Help you optimize recombinant antibody expression; transfection & growth conditions; recombinant antibody purification & characterization.
- Bioinformatics resources available to aid in the design of recombinant antibody experiments

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 more accessible and user-friendly. All key factors have been 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 a higher probability of obtaining functional and active proteins.

Solubility Tags

The *E.coli* expression vector with a unique solubility marker developed by GenScript's professional scientists is specially used for the production of soluble recombinant proteins.

The success rate of the *E. coli* project is greater than 95%.

Contact US and send email to protein@genscript.com

FAQ

Questions related to protein expression

Q: What are the capacities of GenScript's protein expression platforms?

A: For Bacteria System: (a) Provides large quantities of protein, up to grams; (b) Provides large-scale fermentations up to 500 L; (c) Provides homogeneous recombinant proteins, over 98% purity.
For Baculovirus-Insect Cell System: (a) Provides high titer recombinant baculovirus stock carrying the genes of interest, up to 10^8 pfu/mL; (b) Provides homogeneous recombinant proteins, over 98% purity; (c) Provides large-scale protein production, up to hundreds of milligrams (or 100 liter culture).
For Mammalian Cell System: (a) Provides homogeneous recombinant proteins, over 98% purity; (b) Provides protein production, from miligram to kilogram level (or 200 liter culture).

Q: If the expression level of transient expression is low, how do you usually adjust or optimize the experimental protocol to improve the expression level?

A: Generally, the protein expression is mainly determined by the nature of the protein itself. The common optimized measurements are as follows: codon optimization, transfection mode optimization (especially for some cell lines with low transfection efficiency), cell culture scheme optimization, cell lysis buffer testing for protein secretion and expression analysis, host cell line change, signal peptide change, expression vector change, cloning strategy (such as Fc labeling strategy and site mutation), buffer screening of purification steps (precipitation, solubility) and addition of protease inhibitors (if protein degradation occurs).

Q: Why will customers choose GenScript for custom protein expression?

A: GenScript provides a wide range of protein services with the following features: (a) Your custom requests are handled by experienced scientists – we have successfully delivered over 40,000 batches of custom proteins and over 30,000 batches of custom antibodies; (b) High success rate – our *E. coli* protein expression system has a 98% success rate; Our other expression systems also have success rates above the industry average. (c) High capacity – up to 2,000 L bacterial protein production, 100 L insect and 200 L mammalian protein production. (d) GenScript has developed mature platforms with advanced technologies (e.g. OptimumGene™, BacPower™, BacuVance™, and CrystalPro™) to solve your protein puzzles.

Q: Does codon optimization really matter? Is there any difference between codon optimization in gene and protein departments? Does codon optimization promote the solubility of the expressed proteins?

A: Codon optimization can greatly increase the level of protein expression. GenScript OptimumGene™ is the most cited codon optimization technology. Thousands of cases have demonstrated the effectiveness of GenScript's codon optimization.
Currently, the same system is used for codon optimization in both gene and protein departments, and codon optimization only promotes higher protein expression, not solubility.

Q: How much baculovirus would GenScript need to produce the protein if the customer supplied it?

A: If the customers provide baculovirus, they should also provide GenScript with the baculovirus titer and generation of viral stock, MOI, cell density and cell volume for cell infection. We'll use the relevant formula to calculate how much viral stock needs to be added to obtain the inoculums required (mL). MOI is defined as the number of virus particles per cell. Usually the P1 and P2 viral stock is preferred, and the titer of viral stock should be more than 10^7 pfu/mL. If the amount of virus provided is insufficient, we also offer virus amplification service.

Questions related to protein purification

Q: What are the impurities in protein expression? How does GenScript remove these impurities? What are the protein purification methods in GenScript? How does GenScript choose a purification method to use? What is the common process of multi-step purification?

A: Impurities are mostly intracellular or culture medium substances that can be removed by chromatography. GenScript can perform affinity purification, ion exchange chromatography, hydrophobic chromatography and size exclusion chromatography Generally, affinity chromatography is the first choice for proteins containing affinity labels, and then multi-step purification is selected according to the obtained protein purity and the location and properties of impurities .

Q: What does GenScript do during purification if the customer's protein is easily degraded?

A: During the purification process, we will add protease inhibitors and use mild purification methods, such as using cell lysis enzymes instead of ultrasonication, and will operate quickly at low temperatures to purify the protein in a shorter time. If necessary, we will use multi-step purification to obtain higher purity of the target protein.

Q: How does GenScript determine protein purity and concentration?

A: Methods for purity determination: (a) SDS-PAGE (b) SEC-HPLC; SDS-PAGE is used as default.
Methods for protein concentration determination: (a) Branford protein assay; (b) BCA protein assay; Bradford is used as default, and BCA is used if there are any disturbing factors in the buffer. Antibodies are tested with A280.

Q: How are endotoxins controlled? What endotoxin level can GenScript achieve?

A: The prokaryotic system of protein removes endotoxins at three levels: < 1 EU/μg, < 0.1 EU/μg, <0.01 EU/μg. Of which, 0.001 EU/μg is difficult to achieve. The 0.001 EU/g for mammalian expression system is easier to reach, and the antibody can reach 0.0005 EU/μg. The typical endotoxic protein requirement for mice is 0.003 EU/μg, and < 0.001 EU/μg for human. The standard for internal control of mammalian expression is 0.01 EU/mg, but GenScript does not test for endotoxin content by default. If the customer requires final QC data including endotoxin, and if the customer has requirements for endotoxin control, it should be proposed before the experiment. GenScript controls endotoxin levels by managing reagents, containers, to avoid the introduction of endotoxin during expression and purification. If endotoxin levels are still high, further removal can be done with a detoxifying column or ion column.

To help you solve problems related to recombinant protein expression and cell line development, Please visit the FAQs in Technical Resource Center: https://helpcenter.genscript.com/hc/en-us?src=pullmenu, or contact GenScript Professional Support.

Literature Published by Customers

Title: Structure of the human cGAS–DNA complex reveals enhanced control of immune surveillance

Journal: *Cell* IF: 36.216 (2018)

Doi: 10.1016/j.cell.2018.06.026

Introduction: hcGAS (D157–F522) and mcGAS (P147–L507) were codon optimized for bacterial expression.(GenScript)

Title: Ticks Resist Skin Commensals with Immune Factor of Bacterial Origin

Journal: *Cell* IF: 36.216 (2020-12)

Doi: 10.1016/j.cell.2020.10.042

Introduction: 24 h before tick feeding, 0.5 mg affinity purified Rabbit aDae2 custom generated against recombinant Dae2ls protein (GenScript) was diluted in sterile PBS and injected into the intraperitoneal cavity of female C3H/HeJ mice to neutralize against pre-made Dae2ls protein in tick saliva.

Title: Human IFIT3 modulates IFIT1 RNA binding specificity and protein stability.

Journal: *Immunity* IF: 22.845 (2018)

Doi: 10.1016/j.immuni.2018.01.014

Introduction: The coding regions of IFIT1, IFIT2, IFIT3, IFIT5 , ifit1, and ifit3 were codon optimized for expression in E. coli. (GenScript)

Title: Connecting clusters of COVID-19: an epidemiological and serological investigation

Journal: *Lancet Infect Dis* IF: 19.966 (2020-04)

Doi: 10.1016/S1473-3099(20)30273-5

Introduction: For ELISA assays, we used recombinant nucleocapsid protein from SARS-CoV and SARS-CoV-2 expressed in mammalian cell culture using the pcDNA3.1 vector , according to previously published methods and a recombinant receptor binding domain (RBD) of the SARS-CoV-2 spike protein custom-produced by a commercial provider. (GenScript）

Title: Molecularly defined cortical astroglia subpopulation modulates neurons via secretion of Norrin.

Journal: *Nat Neurosci* IF: 17.839 (2019)

Doi: 10.1038/s41593-019-0366-7

Introduction: Recombinant proteins were generated and obtained from GenScript.

Title: CDK7 Inhibition Potentiates Genome Instability Triggering Anti-tumor Immunity in Small Cell Lung Cancer.

Journal: *Cancer Cell* IF: 27.407 (2020)

Doi: 10.1016/j.ccell.2019.11.003 Dec 2019

Introduction: Subsequently, DMSO-conditioned medium or YKL-5-124-conditioned medium were collected and added to above single-cell suspension in a 96-well U-bottom plate in the presence of Ova257–264 peptide (10 mg/mL, GenScript, Cat#RP10611) for 4 days as previously described.

Literature Published by Customers

Title: Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy

Journal: *Sci Transl Med* IF: 16.761 (2020)

Doi: 10.1126/scitranslmed.aav7431 11 Mar 2020

Introduction: After isolation, DCs were either incubated with DQ-OVA (Thermo Fisher Scientific) or pulsed with OVA peptide 257264 SIINFEKL (GenScript).

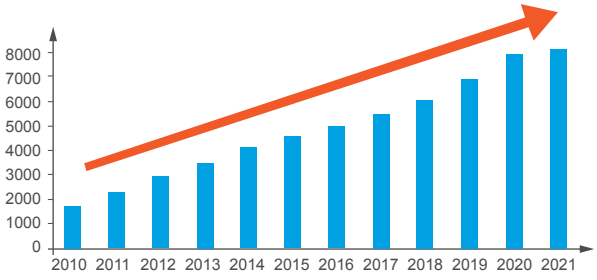
Title: An unbiased approach to defining bona fide cancer neoepitopes that elicit immune-mediated cancer rejection

Journal: *J Clin Invest* IF: 12.784 (2020)

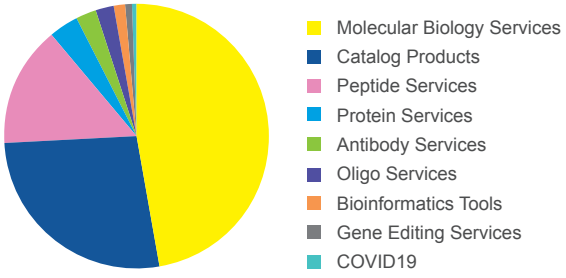
Doi: 10.1172/JCI142823 1 Feb 2021

Introduction: Peptide synthesis Peptides were custom made with a purity of >90% (JPT, Berlin, Germany and GenScript, Piscataway, NJ) and dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 20 mM. Generation of BMDCs and neoepitope vaccine preparation.

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*.
Please visit the customer's published literature at
https://www.genscript.com.cn/reference_peer-reviewed_literature.html.



Number of Literatures cited GenScript from 2010 to 2021



Proportion Distribution of GenScript Products and Services in the Literature

04

Order Method and Order Query

Order Method

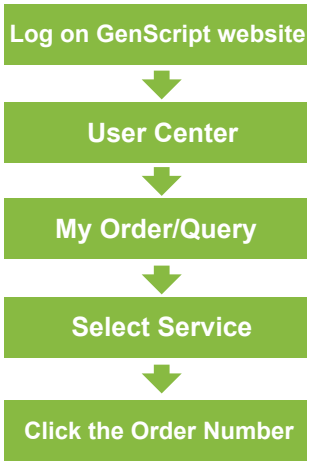
Email US: You can send your demand information to protein@genscript.com

Call US: USA: +1-732-885-9188
Netherlands: +31 (0) 71 569 0120
United Kingdom: +44 (1865) 679988
Singapore: +65 3159 1898
Japan: +81-3-6811-6572
Korea: +82-10-9311-9208

Order Query

How to query?

1. Log in to your GenScript account
2. Click Account Name - User Center
3. Click "My Order/Query" in the taskbar on the left of the page
4. Select "All Types of Orders" in the Order Type
5. Click the order number to enter the "Order Details" page to view the order progress. For delayed or difficult orders, please email us for consultation and confirmation. We will reply and follow up as soon as possible.



For delayed or difficult orders, please email us for consultation and confirmation. We will reply and follow up as soon as possible.

Memo No. _____

Date / /

Memo No. _____

Date / /

GenScript has always been committed to meeting the needs of its customers and to bringing advanced technology to millions of laboratories.

More events
Please follow us on **“GenScript Reagent Service”**