



**Comprehensive CAR-T  
Therapy Development  
Solutions**



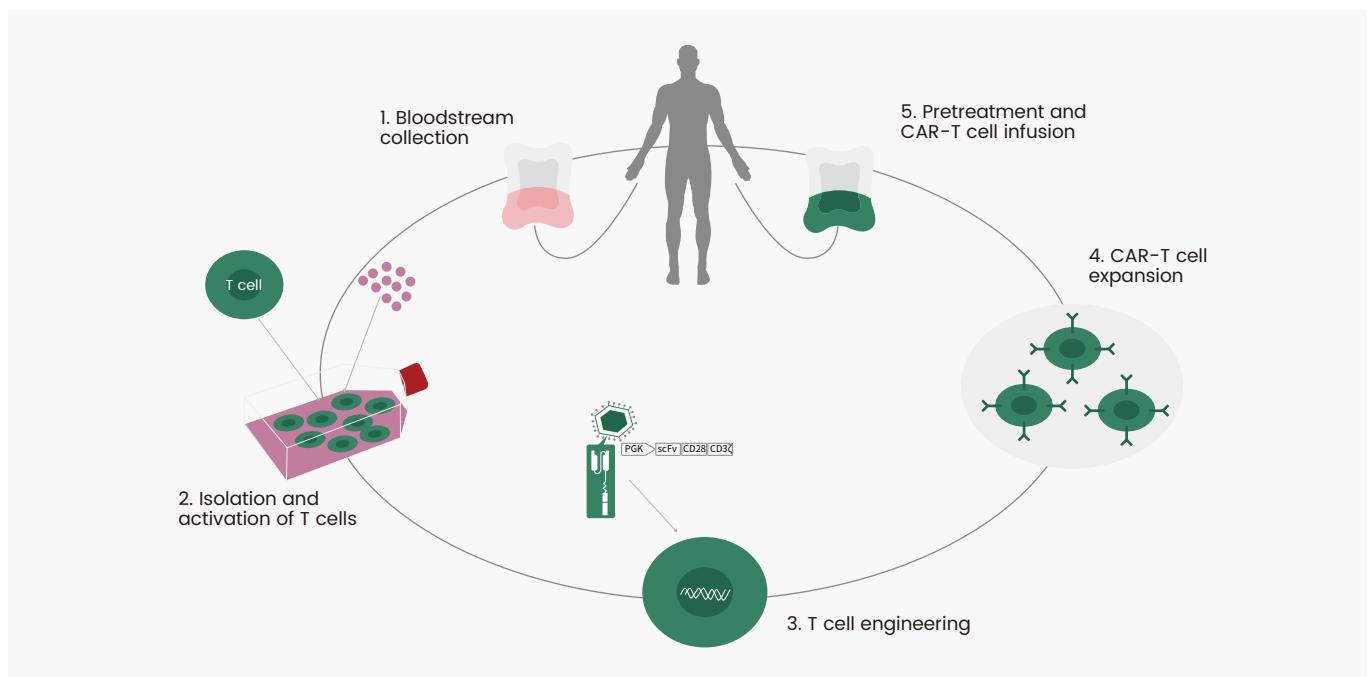
# Introduction to CAR-T Cell Therapy.

## What is CAR-T Cell Therapy

Chimeric antigen receptor T (CAR-T) cell therapy has been rapidly developing in recent years. Unlike traditional cancer therapies, CAR-T therapy is considered as a "living drug" and personalized therapy, which uses the patient's own immune T cells to attack cancer cells.

The entire process of CAR-T therapy can be divided into five steps:

- T cells are collected and isolated from the peripheral blood of the patient (or allogeneic donors).
- Using genetic engineering techniques, T cells are transduced and equipped with a "GPS navigation system" called a CAR, "transforming" T cells into CAR-T cells.
- CAR-T cells are cultured and expanded in vitro to obtain an adequate number of cells for infusion.
- Quality control testing of CAR-T cells.
- The prepared high-quality CAR-T cells are infused into the patient to target and kill cancer cells while avoiding damage to normal tissues.



## FDA-Approved CAR-T Cell Therapies

Since the FDA approval of the first CAR-T cell therapy, Kymriah, in 2017, there are currently six CAR-T drugs approved by the FDA. This innovative cell therapy brought a huge breakthrough in the treatment of malignant hematological tumors, thus, it has led to great interest in R&D for cancer treatment from the research and industry community.

Drug Name	Target	Year	Indication
Kymriah	CD19	2017	B-cell acute lymphoblastic leukemia; Diffuse large B-cell lymphoma (DLBCL)
Yescarta	CD19	2017	Diffuse large B-cell lymphoma (DLBCL); Follicular lymphoma
Tecartus	CD19	2020	Mantle cell lymphoma (MCL)
Breyanzi	CD19	2021	Diffuse large B-cell lymphoma (DLBCL)
Abecma	BCMA	2021	Multiple myeloma
Carvykti	BCMA	2022	Multiple myeloma

# CAR-T Therapy Development Solutions

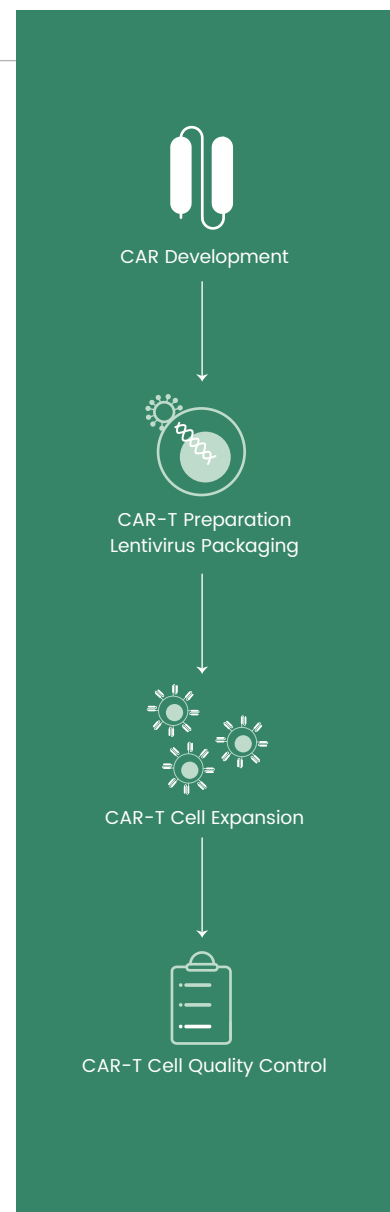
As a global leading supplier of bioreagents and CRO services for the biopharmaceutical field, Sino Biological provides comprehensive solutions for CAR-T cell therapy development. Our reagents and services support clients through each stage from early target discovery to preclinical phase of research and development.

For CAR-T cell therapy, Sino Biological provides comprehensive solutions for pharmaceutical companies from CAR development, T cell activation, lentivirus packaging, CAR-T cell expansion to CAR-T cell quality control, fully supporting CAR-T research.

Covering each step

## Comprehensive Solutions for CAR-T Therapy Development

- | Four Antibody Development Platforms
- | Bioactivity validated CAR-T Target Proteins
- | Premium Cytokines: IL-2, IL-7, IL-15, IL-21
- | Chemically-defined and Serum-free Medium
- | SuperNuclease with Excellent Performance
- | SuperNuclease ELISA Kit



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# CAR Development Solutions

CAR is the core component of CAR-T therapy, and how to obtain CARs that specifically recognize tumor antigens is key to CAR development. Powered by four antibody development platforms such as phage display, hybridoma, FACS single B cell, and Beacon® single B cell, Sino Biological provides different solutions for scFv discovery. To accelerate the process of CAR-T research, we also provides a wide collection of high-quality CAR-T target proteins and professional SPR/BLI affinity characterization services for scFv screening and CAR affinity determination.

## CAR Candidate Development

The extracellular domain of CAR contains monoclonal antibody-derived single-chain variable fragments (scFv) that determines whether CAR-T can specifically recognize tumor cells and plays an important role in the efficacy and safety of CAR-T cells.

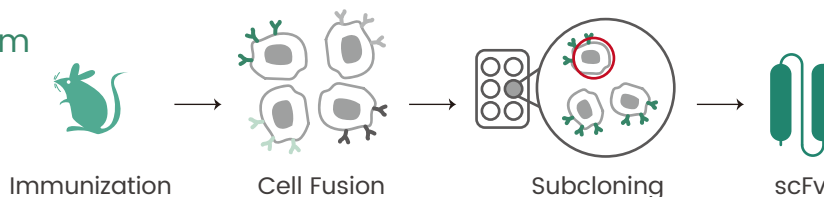
### Four scFv Discovery Platforms

Sino Biological provides diversified scFv development platforms, covering the entire process from antigen design and preparation, animal immunization to obtaining scFv molecules, helping pharmaceutical customers to obtain their proprietary scFv sequences.

#### Diversified Antibody Development Platforms, To Support CAR Candidate Discovery

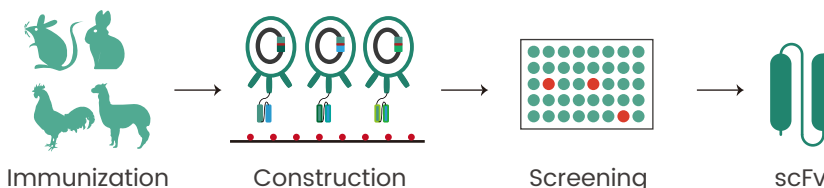
##### Hybridoma Development Platform

- Success rate > 90%
- Well-established technology to generate highly specific scFv molecules



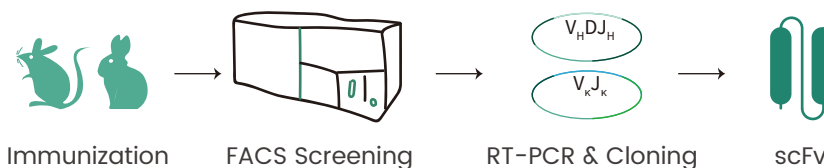
##### Phage Display Platform

- Directly obtain scFv sequences
- HTP screening for scFv molecules



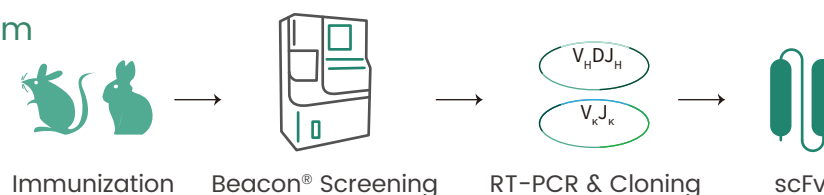
##### FACS B Cell Screening Platform

- All B cells can be sorted in 1 day
- Generating naïve pairing of scFv molecules



##### Beacon® B Cell Screening Platform

- As fast as in 35 days to obtain scFv sequences
- Screening more than 10,000 B cells at one time



## CAR Candidate Screening

Some important characteristics of scFv such as affinity, specificity, and binding epitope may affect the killing ability, persistence, and anti-tumor effect of CAR-T cells. Sino Biological provides a wide range of products and services to fully support the screening of scFv molecules.



scFv Affinity Characterization  
(BLI/SPR)



Target Proteins



Assay Cell Line  
Development



Protein Antigen  
Expression Services

### scFv Affinity Characterization (BLI/SPR)

scFv is critical to specific recognition of tumor cells by CAR-T cells, and its affinity and specificity for antigens affect the efficacy and safety of CAR-T. To support CAR-T drug development, Sino Biological provides SPR/BLI assay platform which can be used to efficiently screen scFv molecules.

#### Service Highlights



Biacore and ForteBio  
Octet analysis platforms



SPR platform is fully  
accredited by CNAS



Authoritative reports support the IND or  
BLA filings of CAR-T drugs

#### Service Items

- Affinity determination
- Epitope analysis
- Consistency evaluation



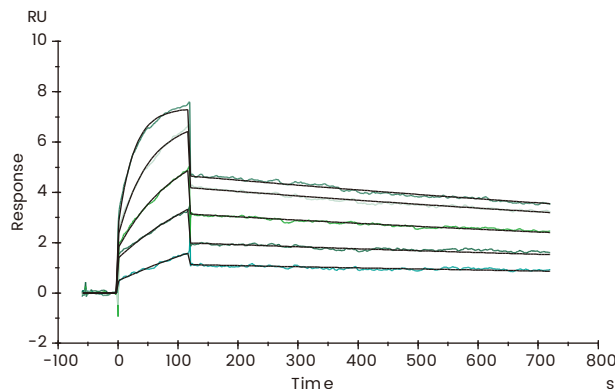
Biacore T200



ForteBio Octet RED384

#### Featured Case Study

Sino Biological used the Biacore platform to detect the affinity between human CD19 protein and scFv, and the affinity constant was  $5.39E-10$  M.



For more service details, please visit <https://www.sinobiological.com/services/spr-bli-assay-services>

## CAR-T Target Proteins

Sino Biological provides hematological tumor and solid tumor target proteins (breast cancer, pancreatic cancer, lung cancer, etc.) with different tags. It can flexibly meet different research and development needs such as scFv screening. The proteins with high purity and high binding activity, have also been validated and cited in high-impact publications. Our expression systems promote proper protein folding and post-translational modifications to serve in your drug discovery programs.

### Advantages



250+ CAR-T Target Proteins  
Various proteins labeling



High bioactivity



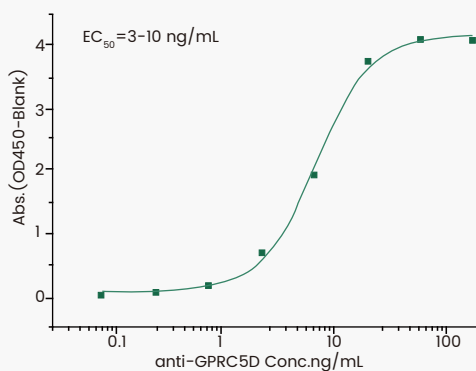
High batch-to-batch consistency

### Multi-pass Transmembrane Proteins

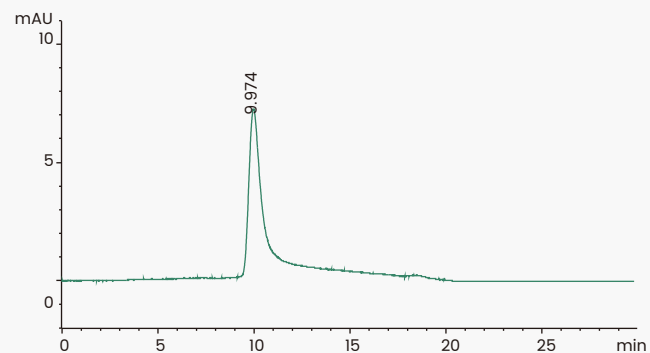
Sino Biological has built multiple technology platforms to address the structural complexity of multi-pass TPs and meet various needs. We have also developed multiple transmembrane target proteins such as GPRC5D, Claudin 18.2 and SSTR2, fully supporting CAR-T drug research.

#### Human GPRC5D-VLP (Full Length) Protein

Cat#: 24447-H18H



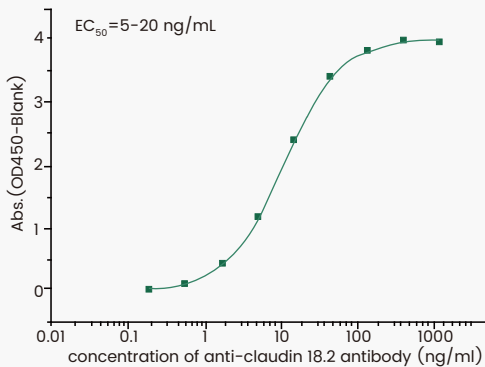
Immobilized Human GPRC5D-VLP (Full Length) Protein (Cat#: 24447-HNAH) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind anti-GPRC5D Antibody, the  $EC_{50}$  is 3-10 ng/mL.



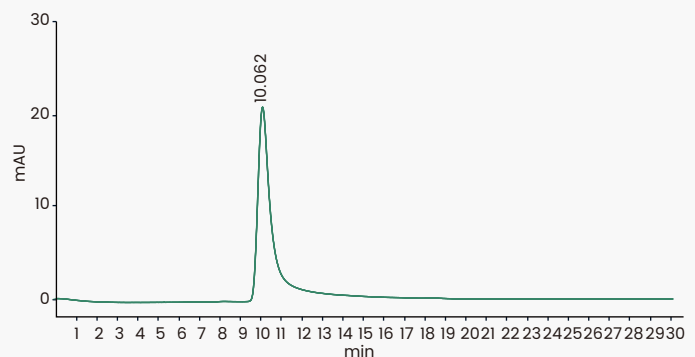
The purity of GPRC5D-VLP (Full Length) Protein is > 95 % as determined by SEC-HPLC.

#### Human Claudin18.2-VLP (Full Length) Protein

Cat#: 30025-HNAH

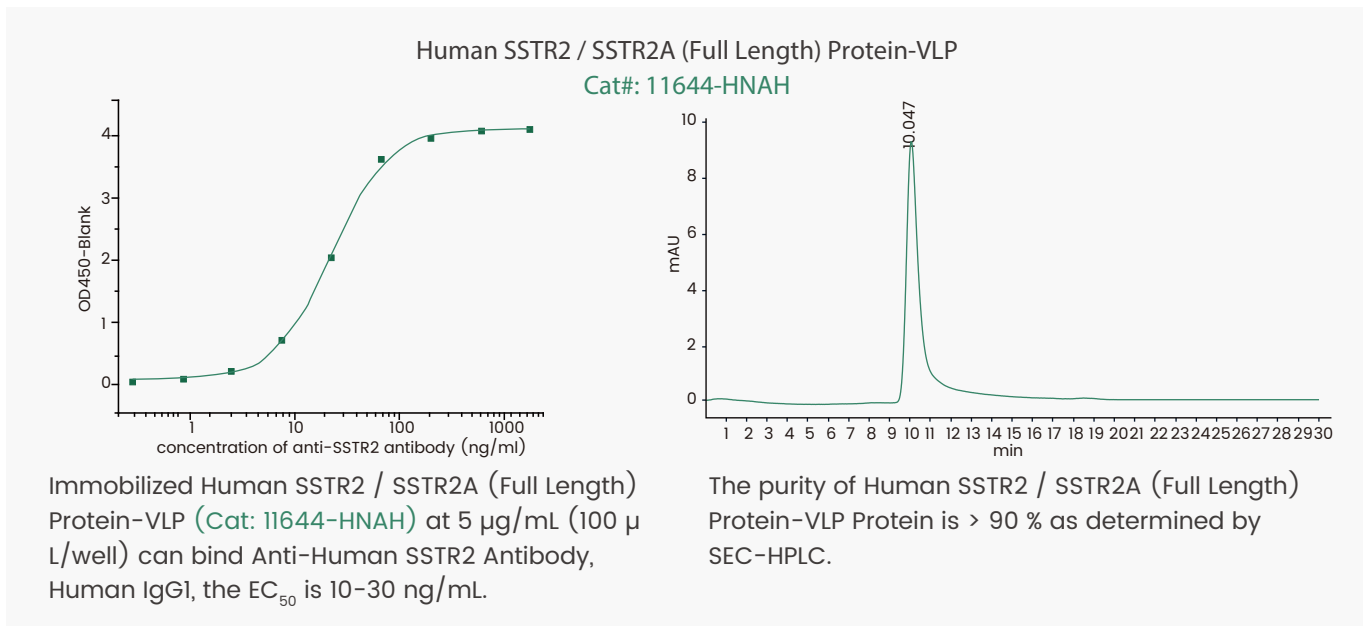


Immobilized Human Claudin18.2-VLP (Full Length) Protein (Cat#: 30025-HNAH) at 5  $\mu$ g/mL (100  $\mu$ L/well) can bind Anti-Human claudin18.2 Antibody, Human IgG1, the  $EC_{50}$  is 5-20 ng/mL.



The purity of Human Claudin18.2-VLP (Full Length) Protein is > 90 % as determined by SEC-HPLC.





**Product List of CAR-T Target Proteins**

**Hematologic Malignancies Target Proteins**

BCMA	CD19	CD20	CD123	CD22
CD3D & CD3E	CD7	CLEC12A	GPRC5D	CD138
CD30	CD33	CD38	CD3E	CD79B
SLAMF7	CD10	CD117	CD37	CD4
CD5	CD56	CD72	CD79A	CD99
FIt-3	LILRA3	LILRB4	SLAMF3	

**Solid Tumors Target Proteins**

Her2	Mesothelin	B7-H3	Claudin 18.2	EGFR
GPC3	KRAS(G12D)	CA9	CEA	EGFRvIII
EphA2	ERBB3	ERBB4	FAP	GUCY2C
IL13RA2	MUC1	PD-1	PSMA	VEGFR2
AFP	AXL	CD133	CD147	CD171
CD80	CD86	c-Met	DLL4	EpCAM
Nectin-4	Podoplanin	ROBO1	ROR2	SSTR2

**CAR-T Target Proteins for both Hematologic Malignancies and Solid Tumors**

FOLR1	ROR1	CD70	NKG2D	PD-L1
SIRP alpha				

For more details, please visit <https://www.sinobiological.com/category/car-t-cell-therapy-target-protein-elite>

## Target Antigen Expression Services

Target antigens are used to determine the specificity and effectiveness of CAR. Sino Biological provides one-stop custom antigen preparation services from gene synthesis, vector construction, protein expression, and purification, fully supporting the development of CAR-T cell therapy.

### Service Highlights

- Multiple protein expression systems
- High-throughput and large-scale protein production
- High-efficiency expression vectors
- Proprietary transfection reagent & medium formulation
- High-density cell culture technology
- Mature technologies for inclusion body refolding & soluble expression

### Popular Protein Expression Systems

HEK293/CHO expression system	Baculovirus-insect expression system	E.coli expression system	Cell-Free protein expression system
			
<ul style="list-style-type: none"> <li>• Flexible cell culture volumes ranging from 1mL to 1500L</li> <li>• 10,000+ successfully completed projects</li> </ul>	<ul style="list-style-type: none"> <li>• Recombinant proteins with a purity of &gt;95%</li> <li>• Successfully completed 1,000+ proteins</li> </ul>	<ul style="list-style-type: none"> <li>• Fast protein delivery within 3 weeks</li> <li>• Successfully completed 1,000+ proteins</li> </ul>	<ul style="list-style-type: none"> <li>• Cell-free reactions are easy to set up and take only one day to express your protein.</li> <li>• Suitable for scFv screening</li> </ul>
			

### Comprehensive Quality Control Systems



SDS-PAGE



ELISA



FACS



SPR



BLI



Cell assay

### Selected Publications Featuring Our Protein Expression Services

Blood. 2009 Jul 9;114(2):310-7. Title: The NKG2D ligand ULBP4 binds to TCRgamma9/delta2 and induces cytotoxicity to tumor cells through both TCRgammadelta and NKG2D. PMID: 19436053. doi: 10.1182/blood-2008-12-196287.

Cell Mol Immunol. 2013 Nov;10(6):463-70. Title: The interaction of influenza H5N1 viral hemagglutinin with sialic acid receptors leads to the activation of human  $\gamma\delta$  T cells. PMID: 23912782. doi: 10.1038/cmi.2013.26.

Cancer Lett. 2013 Dec 1;341(2):150-8. Title: A novel antibody-like TCR $\gamma\delta$ -Ig fusion protein exhibits antitumor activity against human ovarian carcinoma. PMID: 23920126. doi: 10.1016/j.canlet.2013.07.036.

For more service details, please visit <https://www.sinobiological.com/services/recombinant-protein-expression-service>

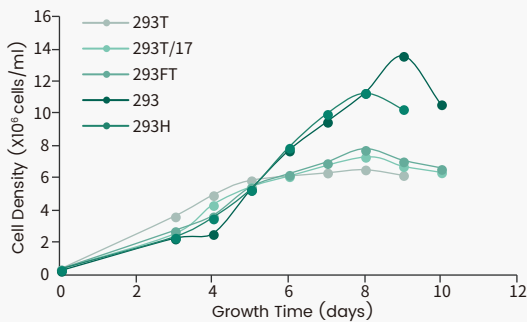
# Lentivirus Packaging Solutions

In CAR-T cell preparation, lentivirus packaging is a key step followed by separation and activation of T cells, and the titer of lentivirus is a key factor in determining the positive rate of CAR transfection. Sino Biological provides key raw materials for lentivirus packaging, such as serum-free HEK293 medium and supplement, as well as SuperNuclease used for nucleic acid removal during the lentivirus purification process. We also provides SuperNuclease ELISA kit for the detection and quantification of residual nuclease impurity.

## Chemically-defined SMM 293-CD1 Medium

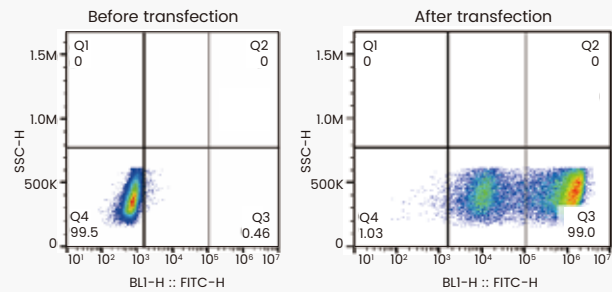
SMM 293-CD1 medium (Cat#: M293CD1) is a ready-to-use medium with well-defined chemical composition, serum-free, no antibiotics, and no animal-derived components, which is suitable for HEK293 cell suspension culture, transient protein expression and lentiviral packaging. SMM 293-CD1 medium is applicable to gene therapy, CAR-T therapy, and other scenarios, successfully supporting customers to complete IND filings.

### Widely applicable to various HEK293 cells



SMM 293-CD1 medium is suitable for suspension culture of various HEK293 cells.

### Efficient and high-density cell transfection



Combined with Sinofection reagent (Cat#: STF02), HEK293 cells were transfected with transfection efficiency of over 95% and transfection density of up to  $3 \times 10^6$ .

### High-density growth with good cell condition



The cells were grown well at high density ( $8 \times 10^6$  cells/mL) in SMM 293-CD1 medium, with no clumping and stable pH.

## Cell Culture Medium Supplement

The cell culture medium supplement SMS 293-SUPI (Cat#: M293-SUPI) developed by Sino Biological is a protein-free, serum-free liquid supplement. It is used for scientific research only and is not recommended for diagnosis and treatment in humans or animals.

Support suspension cell growth

Specifically developed to support the growth of 293E and related cells (such as 293F and 293H.) and supplement nutrients after transient transfection under suspension culture conditions.

Rich in high concentration of nutrients

Contains high concentration of essential amino acids, inorganic salts, and other nutrients required for cell growth.

Maintain high density and high activity of cells

Good stability, promote cell growth, maintain high cell density and cell viability, and greatly improve the yield of proteins and antibodies.

# High-quality SuperNuclease

During the T cell transduction with CAR gene by lentivirus, there will be nucleic acid residues, which are potentially harmful. The residual nucleic acids may cause uncontrolled cell proliferation and conversion into tumor cells in the human body. Therefore, it is very important to use nucleases for effective nucleic acid residue removal.

Sino Biological provides SuperNuclease (Cat#: GMP-SSNP01), which is no animal origin and can efficiently degrade any form of DNA and RNA such as single strand, double strand, linear, circular, and supercoiled structures. SuperNuclease has ultra-high activity, wide application scenarios and has completed FDA DMF filing (DMF Number#: 35978), meeting the requirements for drug declaration.

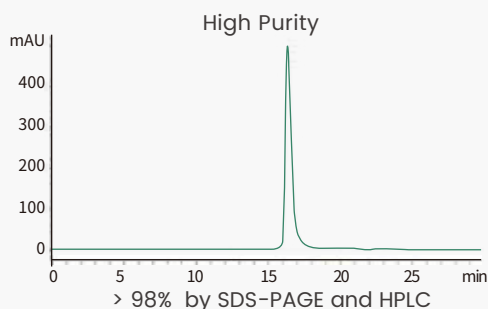
Ultra-high activity

Wide application scenarios

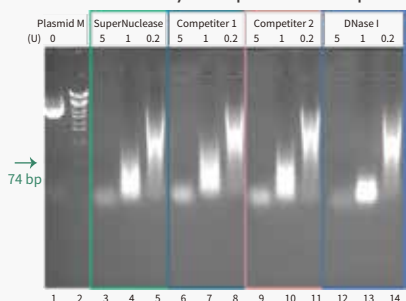
Little amount needed per assay

FDA DMF filed

## High-quality SuperNuclease



The degradation efficiency is equal to competing products.



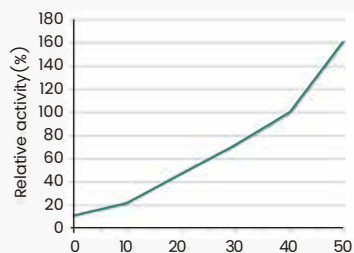
SuperNuclease digestion of plasmid DNA. Different units of SuperNuclease and competitor nucleases were used.

## SuperNuclease under Strict Quality Control Standards

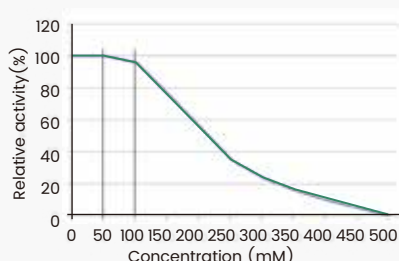
Specifics	Value
Quantity	≥1 mg/ml
Purity	≥95% (SDS-PAGE) ; ≥99% (SEC-HPLC)
Enzyme activity	≥1.1×10 <sup>6</sup> U/mg
Endotoxin	≤10 EU/mg
Proteases	Not detectable
Residual host cell protein	≤10 µg/mg (10000ppm)
Sterility test	No growth
Storage condition	Buffer preparation stored at -20°C to -80°C ; Glycerol preparation stored at -20°C
Storage period	24 months

## SuperNuclease with Excellent Performance

Sino Biological has analyzed the performance of SuperNuclease at various experimental conditions from temperature, Mg ion, pH, buffer, salt concentration and other aspects. According to the following test data, it can be seen that our SuperNuclease has qualifies at basic parameters and shows high stability.



Effect of temperature on SuperNuclease activity



Impact of salt concentration on SuperNuclease activity

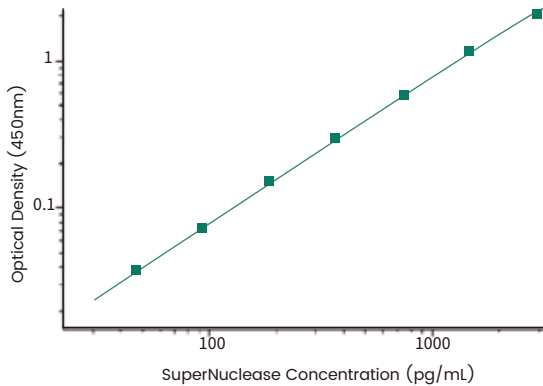
### Excellent performance parameters

Condition	Optimal	Effective
Mg <sup>2+</sup>	1 - 2 mM	1 - 10 mM
pH	8.0 - 9.5	5.5 - 9.5
Temperature	37°C	0 - 50°C
DTT	0 - 200 mM	>200 mM
Monovalent cation concentration (Na <sup>+</sup> )	0 - 100 mM	0 - 400 mM
PO <sub>4</sub> <sup>3-</sup>	0 mM	0 - 50 mM
Tween 20	0 - 0.8%	>0.8%
Brij 35	0 - 0.8%	>0.8%

# SuperNuclease Detection ELISA Kit

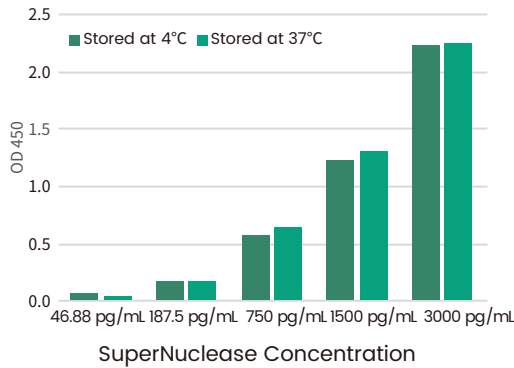
After effective removal of residual nucleic acids by nucleases, nucleases also should be removed from the final product. Any trace residues will impact the safety of CAR-T cells. It is essential to detect residual nucleases to ensure the quality of CAR-T products. Sino Biological provides SuperNuclease ELISA Kit (Cat#: KIT-SSNP01) for the detection and quantitative determination of SuperNuclease and other mainstream nucleases on the market. The kit has a wide determination range from 46.87 to 3000 pg/mL, with high detection accuracy, recovery rate of 80% to 120%, high sensitivity, and the lowest detection limit of 14.55 pg/mL.

## High sensitivity

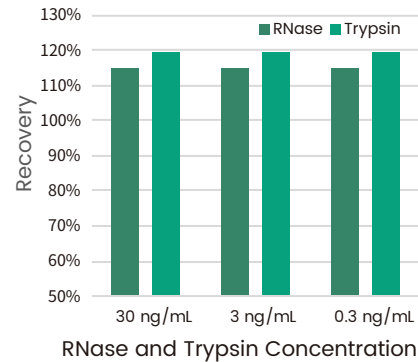


KIT-SSNP01	
Assay range	46.87-3000 pg/mL
Accuracy	Recovery: 80%-120%
Precision	CV (<15%)
Sensitivity	14.55 pg/mL

## Stable and reliable quality



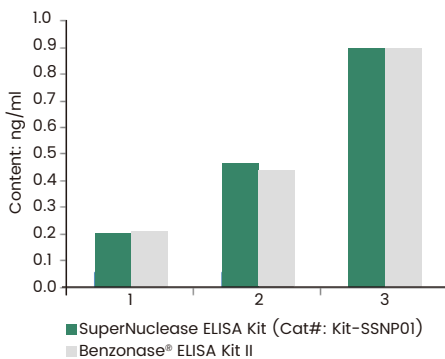
Detection of nuclease by SuperNuclease ELISA kit stored at different temperatures



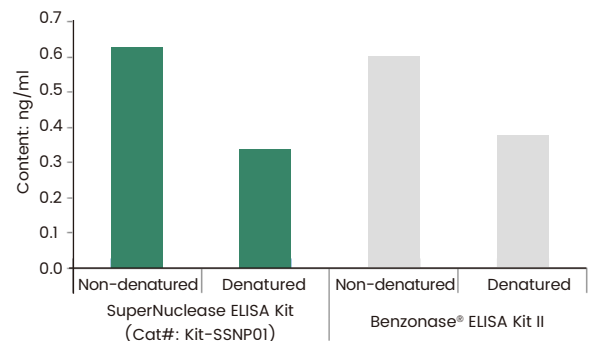
Detection of RNase and trypsin by SuperNuclease ELISA kit with different interferences

## Good versatility

The accuracy of SuperNuclease ELISA Kit is comparable to other brand.



Detection of residual SuperNuclease by SuperNuclease ELISA Kit and Benzonase® ELISA Kit II



Detection of the content of non-denatured and denatured Benzonase® by SuperNuclease ELISA Kit and Benzonase® ELISA Kit II

Lentivirus Packaging

# CAR-T Cell Expansion Solutions

CAR-T cell therapies have generated considerable enthusiasm in the oncology community since the first FDA approval in 2017. Two CAR-T cell products aimed to target CD19 and BCMA were approved by FDA for treating relapsed/refractory set of cell lymphoma or multiple myeloma. During the process of CAR-T cell therapy products, cytokines such as IL-7 and IL-21 can regulate the amplification, sustain activation and anti-tumor capacity of CAR-T cells, and are widely used in the preparation and culture of CAR-T cells.

Sino Biological has successfully developed a series of high quality cytokines such as IL-2, IL-7, IL-15 and IL-21 to facilitate the development of cell therapy drugs.

## Featured Cytokines

CAR-T cells can specifically recognize tumor antigens to exert their anti-tumor activity. Related studies have shown that combined or single use of cytokines can enhance the proliferation of CAR-T cells and improve the anti-tumor activity. Therefore, selecting different cytokine combinations or appropriate cytokines to culture T cells can significantly increase the efficacy of CAR-T immune cells. The following table shows the effect of different cytokines in CAR-T cell therapy drug development.

Effect of cytokines in CAR-T cell therapy drug development

Cytokines	Function	References
IL-2	To promote proliferation of T cells	Am J Cancer Res. 2020 Dec 1;10(12):4038-4055.
IL-7	To improve anti-tumor activity of CAR-T cells	Blood. 2010 Apr 29;115(17):3508-19.
IL-15	To improve anti-tumor activity of CAR-T cells and enhance CAR-T cell proliferation	Cancer Immunol Res. 2019 May;7(5):759-772.
IL-21	To improve anti-tumor activity of CAR-T cells and promote T cell proliferation	J Immunol. 2004 Jul 15;173(2):900-9.
IL-7+IL-15	To enhance anti-tumor activity of CAR-T cells and function of T memory cells	Blood. 2014 Jun 12;123(24):3750-9.
IL-15+IL-21	To enhance anti-tumor activity of CAR-T cells and function of T memory cells	Hum Gene Ther Methods. 2014 Dec;25(6):345-57.

Sino Biological developed a series of high-quality cytokines IL-2, IL-7, IL-15 and IL-21 for CAR-T cell culture.



High Purity



HPLC Verified



Activity Validated



High Lot-to-lot  
Consistency



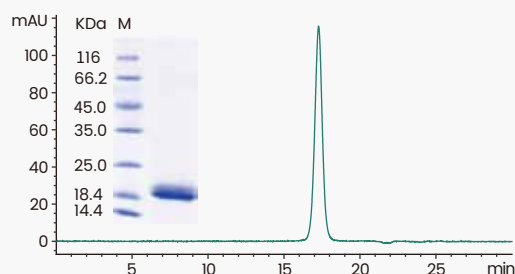
Low-endotoxin



Cited by Researchers

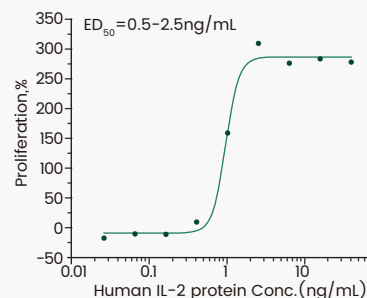
### Human IL-2 (Cat#: 11848-HNAH1-E)

#### High Purity



Purity:  $\geq 95\%$  as determined by SDS-PAGE & HPLC

#### High Bioactivity



Cell proliferation assay using CTLL-2 mouse cytotoxic T cells

### Human IL-7 (Cat#: 11821-HNAE)

**High Purity**

Purity: ≥95% as determined by SDS-PAGE & HPLC

**High Bioactivity**

ED<sub>50</sub>=0.5-8ng/mL

Cell proliferation assay using anti-CD3 antibody activated human peripheral blood mononuclear cell.

**High Batch to Batch Consistency**

Batch-to-batch Consistency

### Human IL-15 (Cat#: 10360-HNCE)

**High Purity**

Purity: ≥95% as determined by SDS-PAGE

**High Bioactivity**

EC<sub>50</sub>=2-8ng/mL

Cell proliferation assay using MO7e human megakaryocytic leukemic cells

**High Affinity Validated by BLI**

Using the Octet RED System, the affinity constant (Kd) of IL-15 Protein, Human, Recombinant, Biotinylated bound to Anti-IL15 Antibody was 0.1nM.

### Human IL-21 (Cat#: 10584-HNAE)

**High Purity**

Purity: ≥95% as determined by SDS-PAGE & HPLC

**High Bioactivity**

ED<sub>50</sub>=0.4-2ng/mL

Ability to induce Interferon-gamma secretion by human natural killer lymphoma NK-92 cells

**High Batch to Batch Consistency**

Batch-to-batch Consistency

CAR-T Cell Expansion

## List of More Cytokines

In addition, for the large-scale culture of immune cells such as CAR-T/NK and various types of stem cells, Sino Biological has developed more cytokines to facilitate cell therapy research!

Molecule	Cat#	Species	Purity	Bioactivity (ED <sub>50</sub> )
bFGF/FGF2	10014-HNAE	Human	95%	0.02-0.1 ng/mL
FGF6	11528-HNAE	Human	95%	1-6 ng/mL
IL3	11858-HNAE	Human	95%	0.1-0.6 ng/mL
IL4	11846-HNAE	Human	95%	0.05-0.25 ng/mL
IL6	10395-HNAE	Human	95%	0.1-0.8 ng/mL
IL7	11821-HNAE	Human	95%	0.5-8 ng/mL
IL10	10947-HNAE	Human	95%	0.5-2.5 ng/mL
EGF	10605-HNAE	Human	95%	0.02-0.2ng/mL

# CAR-T Cell Quality Control Solutions

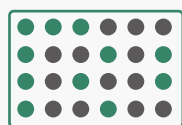
The quality of CAR-T cells directly affects the clinical treatment effect and patients' health. It may even endanger patients' lives if not strictly tested. Therefore, CAR-T cells require strict release testing before infused back into patients. The quality control testing of CAR-T cells usually includes: 1) CAR-T cell purity testing; and 2) CAR-T cell potency and functionality testing.

## CAR-T Cell Purity Testing

Test Items	Purpose	Solutions
Cell Population Profiling	Monitoring CAR-T cell differentiation, exhaustion, apoptosis, etc.	A wide range of FACS antibodies, used in: <ul style="list-style-type: none"> <li>• CD3+ T cell percentage, CD4+/CD8+ percentage, etc.</li> <li>• Immunophenotyping: <math>T_n</math>, <math>T_{scm}</math>, <math>T_{cm}</math>, <math>T_{em}</math></li> <li>• Activation markers: CD25, CD69</li> <li>• Exhaustion markers: PD-1</li> </ul>
Detection of Target Cells	Understanding key features of CAR-T products	<ul style="list-style-type: none"> <li>• 250+ CAR-T target proteins: high specificity, covering fluorescent, site-specific biotinylated, unconjugated formats</li> <li>• Protein L: universal detection and low cost</li> <li>• Anti-idiotypic antibody services: specifically designed for scFv, with good specificity</li> </ul>
Detection of Non-Target Cells	Detecting percentage of residual tumor cells	High-quality CAR-T target antibodies, covering CD19, CD20, CD22, EGFR, and CD3 (fluorescence conjugated versions available)
Test of Impurities	Detecting residual cytokines	IL-2 ELISA Kit (Cat#: KIT11848), IL-7 ELISA Kit (Cat#: KIT11821)

## CAR-T Potency and Functionality Testing

The functional testing of CAR-T cells is an important step in the quality control of CAR-T cells, including cytokine release testing, in vitro killing ability, and intracellular signal transduction analysis. Sino Biological has developed high-quality cytokine ELISA kits, which can be used to detect the levels of representative cytokines to evaluate the killing activity and specificity of CAR-T cells on target cells. Furthermore, we also construct stable tumor cell lines, which can be used to evaluate the killing activity of CAR-T cells in vitro.



ELISA Kits

- 600+ kits, including human (400+) and mouse (100+)
- Strictly follow eight QC standards to ensure the validity and reliability of the assay results.
- High sensitivity and specificity, good stability and reproducibility



Assay Cell Line Development

- 15+ years of experience in cell line development
- Fast delivery of stable cell lines in 2-4 months
- Complete platform with FACS, qPCR, WB and other identification methods



# CAR-T Cell Purity Testing (Cell Population Profiling)

## High-quality FACS Antibodies

When the immune cell therapy product is a mixture of multiple different types or different genotypes/phenotypes of cells, it is recommended to investigate the composition and proportion of cell population or subpopulation in the sample. For example, cell population profiling can be used to monitor the differentiation, exhaustion, and apoptosis of CAR-T cells. Flow cytometry is a key method to determine the components of CAR-T cells, and the quality of flow cytometry antibodies directly affects the accuracy of detection results.

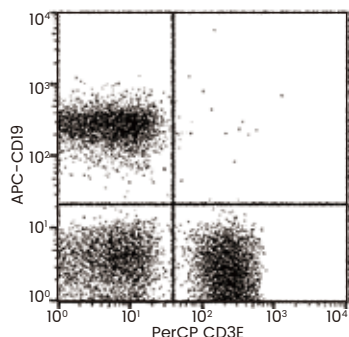
Sino Biological has independently developed a variety of flow cytometry antibodies. The flow antibodies are immunized with recombinant proteins expressed in eukaryotic cells. With good specificity, affinity and signal-to-noise ratio, these antibodies ensure accurate detection.

- High specificity
- High affinity
- High signal-to-noise ratio
- Multiple Conjugates

## Percentage of CD3+, CD4+, and CD8+ T cells

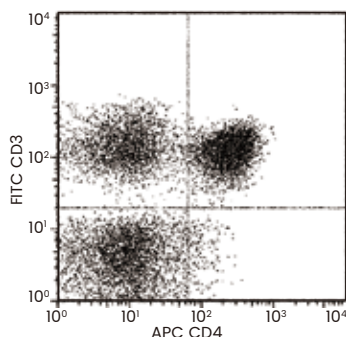
The detection of T lymphocyte populations is one of the latest indicators for cellular immune detection, and flow cytometry is used to detect the levels of different T lymphocyte populations or subpopulations. T cell subpopulations with different functions have their own marker antigen, and T cells can be divided into CD3+CD4+CD8- helper T cells (Th), CD3+CD4-CD8+ cytotoxic T cells (Tc or CTL) and CD4+CD25+ regulatory T cells (Tr or Treg) according to the expression of T cell surface antigens. Sino Biological has developed a variety of labeled CD3, CD4, CD8 FACS antibodies to help with efficient identification of the modified T cell populations or subpopulations.

CD3D & CD3E Antibody (PerCP),  
Rabbit MAb (Cat#: CT026-R301-C)



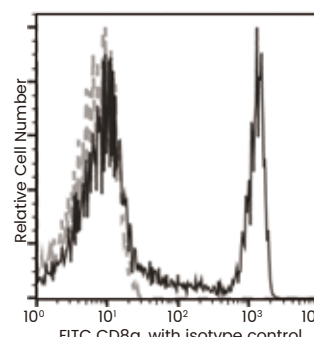
Flow cytometric analysis of Human CD3 expression on human peripheral blood lymphocytes.

CD4 Antibody (APC), Mouse MAb  
(Cat#: 10400-MM08-A)



Flow cytometric analysis of Human CD4 expression on human peripheral blood lymphocytes.

CD8 Antibody (FITC), Mouse MAb  
(Cat#: 10980-MM48-F)



Flow cytometric analysis of Human CD8a expression on human whole blood lymphocyte.

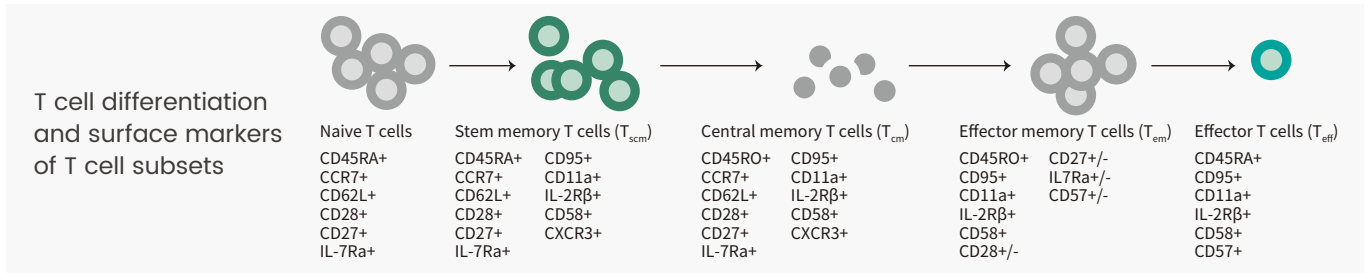
## List of More FACS Antibody Products

Antigen	Product Name	Ab Type	Species	Cat (Conjugate)
CD3	CD3D & CD3E Antibody	Rabbit MAb	Human	CT026-R301 (Unconjugated)
				CT026-R301-A (APC)
				CT026-R301-C (PerCP)
				CT026-R301-F (FITC)
				CT026-R301-P (PE)
CD4	CD4 Antibody	Mouse MAb	Human	10400-MM08 (Unconjugated)
				10400-MM08-A (APC)
				10400-MM08-C (PerCP)
				10400-MM08-F (FITC)
				10400-MM08-P (PE)
CD8	CD8 alpha/CD8A Antibody	Mouse MAb	Human	10980-MM48 (Unconjugated)
				10980-MM48-A (APC)
				10980-MM48-C (PerCP)
				10980-MM48-F (FITC)
				10980-MM48-P (PE)

CAR-T Cell Quality Control

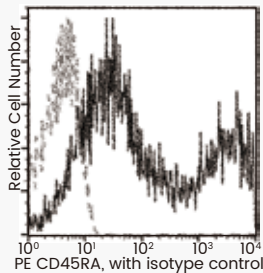
$T_n, T_{scm}, T_{cm}, T_{em}$  Immunophenotyping

According to the different surface markers, monoclonal antibodies are used to identify T cells at diverse differentiation stages. Sino Biological has developed a variety of CD45RA, CD27, CD95, CD62L, CD58, IL-17RA FACS antibodies with different conjugates to help you efficiently identify specific immune cell populations and subpopulations.



CD45RA Antibody (PE), Mouse MAb

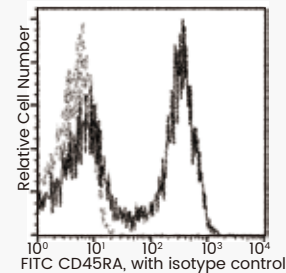
(Cat#: 68036-XM01-P)



Flow cytometric analysis of Human CD45RA expression on human whole blood lymphocytes. Cells were stained with PE-conjugated anti-Human CD45RA.

CD45RA Antibody (FITC), Mouse MAb

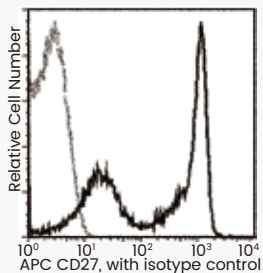
(Cat#: 68036-XM01-F)



Flow cytometric analysis of Human CD45RA expression on human whole blood lymphocytes. Cells were stained with FITC-conjugated anti-Human CD45RA.

CD27 Antibody (APC), Mouse MAb

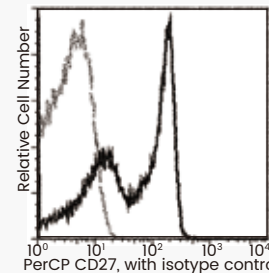
(Cat#: 10039-MM09-A)



Flow cytometric analysis of anti-human CD27 on human whole blood lymphocytes.

CD27 Antibody(PerCP), Mouse MAb

(Cat#: 10039-MM09-C)



Flow cytometric analysis of anti-human CD27 on human whole blood lymphocytes.

List of More Immunophenotyping Antibody Products

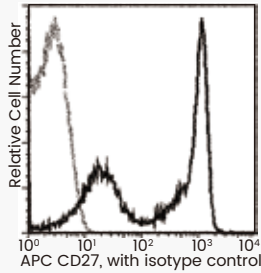
Antigen	Ab Type	Species	Cat (Conjugate)	Antigen	Ab Type	Species	Cat (Conjugate)
CD45RA	Mouse MAb	Human	68036-XM01-F (FITC)	CD27	Mouse MAb	Human	10039-MM09 (Unconjugated)
			68036-XM01-P (PE)				10039-MM09-A (APC)
CD95	Mouse MAb	Human	10217-MM10 (Unconjugated)	CD62L	Mouse MAb	Human	11838-MM01 (Unconjugated)
			10217-MM10-A (APC)				11838-MM01-A (APC)
			10217-MM10-F (FITC)				11838-MM01-C (PerCP)
			10217-MM10-P (PE)				11838-MM01-P (PE)
CD58	Rabbit MAb	Human	12409-R083 (Unconjugated)	IL-17Ra	Mouse MAb	Human	10895-MM06 (Unconjugated)
			12409-R083-C (PerCP)				10895-MM06-A (APC)
			12409-R083-F (FITC)				10895-MM06-F (FITC)
			12409-R083-P (PE)				10895-MM06-P (PE)

### T Cell Activation Marker Detection

During the activation stage, T lymphocytes express different activation molecules on the cell surface, which are the criteria to determine whether T lymphocytes are effectively activated. CD69 and CD25 are markers of T cell activation. The surface glycoprotein CD69 is expressed at very low levels in quiescent lymphocytes and increases in a time-dependent manner once T cells are activated. CD25 is the  $\alpha$  chain of IL-2 receptor, which can be used to detect T cell activity and effectively reflect the degree of T cell activation. Sino Biological has developed CD69 and CD25 flow cytometry antibodies to help detect the activation level of T cells.

#### CD69 Antibody (APC), Mouse MAb

(Cat#: 11150-MM06-A)



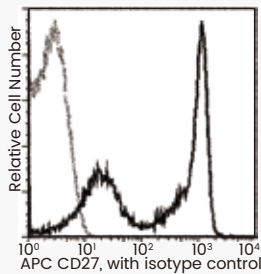
Flow cytometric analysis of Human CD69 expression on PHA-activated human whole blood lymphocytes.

#### List of CD69 Monoclonal Antibody Products

Antigen	CD69
Ab Type	Mouse MAb
Species	Human
Cat (Conjugate)	11150-MM06-A (APC) 11150-MM06-C (PerCP) 11150-MM06-F (FITC) 11150-MM06-P (PE)

#### CD25/IL2R alpha Antibody (APC), Mouse MAb

(Cat#: 10165-MM17-A)



Flow cytometric analysis of IL2RA (CD25) expression on stimulated human peripheral blood lymphocytes.

#### List of CD25 Monoclonal Antibody Products

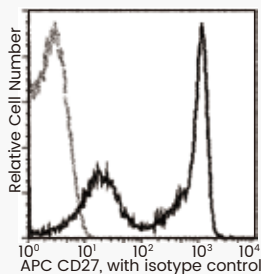
Antigen	CD25
Ab Type	Mouse MAb
Species	Human
Cat (Conjugate)	10165-MM17 (Unconjugated) 10165-MM17-A (APC) 10165-MM17-C (PerCP) 10165-MM17-F (FITC) 10165-MM17-P (PE)

### T Cell Exhaustion Marker Detection

Under consistent antigen/inflammation exposure, T cells will gradually lose the effector function; the characteristics of memory T cells also start to lose, and T cells eventually remain in a state of functional exhaustion. This process is called T cell Exhaustion. For effective immunotherapy, it is necessary to understand the exhaustion state of CAR-T cells. Overexpression of PD-1 is one of the indicators of T cell exhaustion, and T cells with highly expression of PD-1 will eventually enter the end of exhaustion. If the proportion of CD8+T cells with exhausted phenotype (PD-1, LAG-3) increases, it may lead to CAR-T treatment failure. Sino Biological has independently developed PD-1 flow cytometry antibodies, to track the depletion state of T cells.

#### PD-1/PDCD1 Antibody (APC), Mouse MAb

(Cat#: 10377-M140-A)



Flow cytometric analysis of Human PD-1 (CD279) expression on PHA-activated human whole blood Lymphocytes.

#### List of PD-1 Monoclonal Antibody Products

Antigen	PD-1
Ab Type	Mouse MAb
Species	Human
Cat (Conjugate)	10377-M140 (Unconjugated) 10377-M140-A (APC) 10377-M140-C (PerCP) 10377-M140-F (FITC) 10377-M140-P (PE)

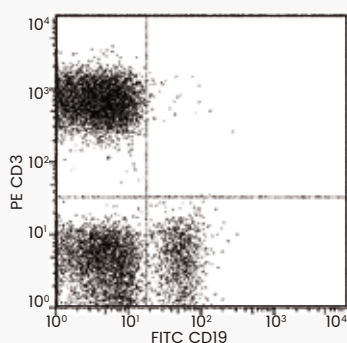
# CAR-T Cell Purity Testing (Detection of Non-target Cells)

## Target Antibodies

The proportion of non-target cells is detected to improve product quality. Therefore, the purity study of CAR-T cells should also include qualitative and/or quantitative studies of non-target cells. For example, the residual non-target cells such as tumor cells, and iPS cells have a high safety risk, so it is necessary to study its proportion and strictly control it. If the non-target cells do not affect the safety and effectiveness of the product, it is necessary to study their composition and proportion, and control batch-to-batch consistency. Sino Biological provides high-quality CAR-T target antibody products, covering CD19, CD20, CD22, Her2, TROP2 and other popular target antibodies for hematological and solid tumors.

## Hematology Tumor Target Antibodies

CD19 Antibody (FITC), Mouse MAb  
(Cat#: 11880-MM17-F)



Flow cytometric analysis of Human CD19 expression on human whole blood lymphocytes.

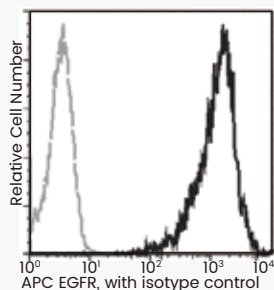
List of CD19 Monoclonal Antibody Products

Antigen	CD19
Ab Type	Mouse MAb
Species	Human
Cat (Conjugate)	11880-MM17 (Unconjugated) 11880-MM17-F (FITC) 11880-MM17-P (PE)

## Partial List of Hematology Tumor Target Antibodies

Antigen	Ab Type	Species	Cat (Conjugate)	Antigen	Ab Type	Species	Cat (Conjugate)
MS4A1/ CD20	Rabbit MAb	Human	11007-R001 (Unconjugated) 11007-R001-A (APC) 11007-R001-C (PerCP) 11007-R001-F (FITC) 11007-R001-P (PE)	CD22	Mouse MAb	Human	11958-MM13 (Unconjugated) 11958-MM13-C (PerCP) 11027-R028-F (FITC) 11027-R028-P (PE)
NCAM1	Mouse MAb, Rabbit MAb	Human	10673-MM01 (Unconjugated) 10673-MM01-A (APC) 10673-MM01-F (FITC) 10673-MM05-F (FITC) 10673-MM05-P (PE)	IL3Ra	Mouse MAb	Human	10518-MM57 (Unconjugated) 10518-MM57-A (APC) 10518-MM57-C (PerCP) 10518-MM57-F (FITC) 10518-MM57-P (PE)
CD38	Mouse MAb	Human	10818-MM27 (Unconjugated) 10818-MM27-A (APC) 10818-MM27-C (PerCP) 10818-MM27-F (FITC) 10818-MM27-P (PE)	TNFRSF8	Mouse MAb, Rabbit MAb	Human	10777-MM05 (Unconjugated) 10777-MM05-A (APC) 10777-MM05-F (FITC) 10777-MM05-P (PE)
CD33	Mouse MAb, Rabbit MAb	Human	12238-MM06-F (FITC) 12238-R001-A (APC) 12238-R001-C (PerCP) 12238-R001-F (FITC) 12238-R001-P (PE)	CD5	Rabbit MAb	Human	11027-R028 (Unconjugated) 11027-R028-A (APC) 11027-R028-C (PerCP) 11027-R028-F (FITC) 11027-R028-P (PE)
CD7	Mouse MAb	Human	11028-MM12 (Unconjugated) 11028-MM12-A (APC) 11028-MM12-F (FITC) 11028-MM12-P (PE)	CD3E	Mouse MAb	Human	10977-M001 (Unconjugated) 10977-M001-A (APC) 10977-M001-C (PerCP) 10977-M001-F (FITC) 10977-M001-P (PE)

## Solid Tumor Target Antibodies

EGFR Antibody (APC), Mouse MAb  
(Cat#: 10001-MM08-A)

Flow cytometric analysis of EGFR expression on human A431 cells.

## List of EGFR Monoclonal Antibody Products

Antigen	EGFR
Ab Type	Mouse MAb, Rabbit MAb
Species	Human
Cat (Conjugate)	10001-MM08-A (APC) 10001-MM08-F (FITC) 10001-MM08T-P (PE) 10001-RE01-A (APC) 10001-RE01-F (FITC) 10001-RE01-P (PE)

## Partial List of Solid Tumor Target Antibodies

Antigen	Ab Type	Species	Cat (Conjugate)	Antigen	Ab Type	Species	Cat (Conjugate)
EGFR	Mouse MAb, Rabbit MAb	Human	10001-MM08-A (APC) 10001-MM08-F (FITC) 10001-RE01-A (APC) 10001-RE01-F (FITC)	ERBB2/ Her2	Mouse MAb, Rabbit MAb	Human	10004-MM07T (Unconjugated) 10004-MM07T-A (APC) 10004-MM07T-F (FITC) 10004-R511-A (APC) 10004-R511-F (FITC)
TROP2 / TACSTD2	Mouse MAb, Rabbit MAb	Human	10428-MM01-F (FITC) 10428-MM01-P (PE) 10428-R001-A (APC) 10428-R001-F (FITC) 10428-R001-P (PE)	GPC3	Rabbit MAb	Human	100393-R024-A (APC) 100393-R024-F (FITC) 100393-R024-P (PE)
CD274	Mouse MAb, Rabbit MAb	Human	10084-R312 (Unconjugated) 10084-R312-A (APC) 10084-R312-F (FITC) 10084-R312-P (PE)	IL13Ra2	Rabbit MAb	Human	10350-R018 (Unconjugated) 10350-R018-A (APC) 10350-R018-F (FITC) 10350-R018-P (PE)
PDCDI	Mouse MAb	Human	10377-M140 (Unconjugated) 10377-M140-A (APC) 10377-M140-C (PerCP) 10377-M140-F (FITC) 10377-M140-P (PE)	MET	Rabbit MAb	Human	10692-R271 (Unconjugated) 10692-R271-A (APC) 10692-R271-F (FITC) 10692-R271-P (PE)
EpCAM	Mouse MAb, Rabbit MAb	Human	10694-MM06 (Unconjugated) 10694-MM06-A (APC) 10694-MM06-F (FITC) 10694-MM06-P (PE) 10694-R028-A (APC) 10694-R028-F (FITC)	CEACAM5	Mouse MAb, Rabbit MAb	Human	11077-R061 (Unconjugated) 11077-MM02-P (PE) 11077-R061-A (APC) 11077-R061-F (FITC) 11077-R061-P (PE)
CD276	Mouse MAb, Rabbit MAb	Human	11188-MM06-A (APC) 11188-MM06-F (FITC) 11188-MM06-P (PE)	ERBB3	Mouse MAb	Human	10201-MM03-P (PE)

For more details, please visit <https://www.sinobiological.com/research/car-t-therapy/car-t-cell-purity-detection>

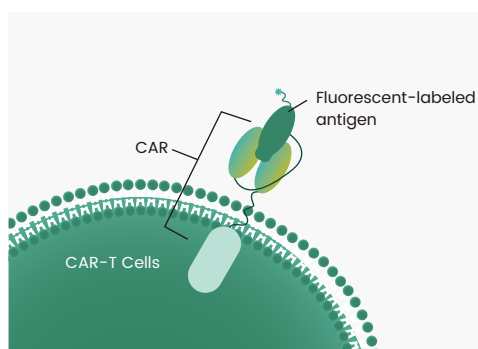
## Purity detection of CAR-T cells (Detection of Target Cells)

Efficient killing of tumor cells by CAR-T cells are required for their quantity and ratio. From preclinical research to post-marketing follow-up, CAR+% detection is inevitable. Therefore, testing the positive rate of CAR expression is an essential step for CAR-T quality control. A wide variety of CAR T cell detection methods have been described so far, such as protein L, anti-Fab antibodies or target antigens. Among them, target antigens are widely used because of its high specificity and minimal background staining.

Sino Biological has successfully developed recombinant proteins, covering most of the investigational CAR-T cell therapy targets. We have developed biotinylated and fluorescent-label proteins to support your scientific research.

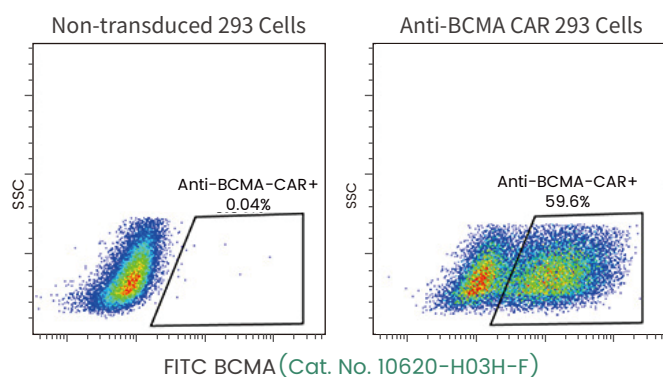
### CAR-T Target Proteins

#### Fluorescent-labeled Target Proteins



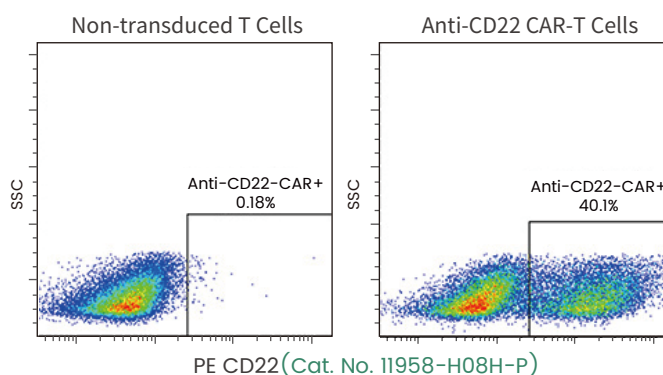
- Avoiding background staining caused by secondary antibody cross-reaction
- Highlighting popular target antigens related with current CAR-T research
- providing one-stop solution to satisfy customized services for multiple labeling approaches
- Featured with high bio-activity, good uniformity, and high batch-to-batch consistency

FACS analysis of anti-BCMA CAR expression  
with FITC-labeled BCMA  
(Cat#: 10620-H03H-F)



293 cells were lentivirally transduced with anti-BCMA CAR. Flow cytometric analysis was performed with FITC-conjugated recombinant human BCMA (Cat#: 10620-H03H-F). Non-transduced 293 cells were used as a control (left).

FACS analysis of anti-CD22 CAR expression  
with PE-labeled CD22  
(Cat#: 11958-H08H-P)



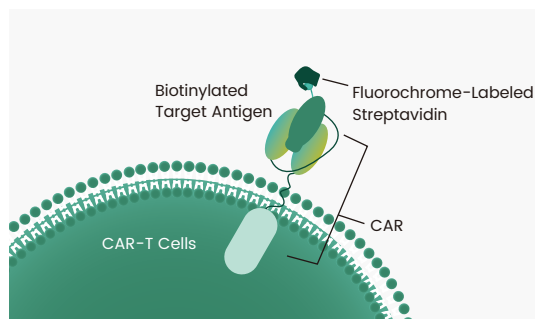
Human T cells were lentivirally transduced with anti-CD22 CAR. Flow cytometric analysis was performed with PE-conjugated recombinant human CD22 (Cat#: 11958-H08H-P). Non-transduced T cells were used as a control (left).

#### Product List of Fluorescent-labeled Proteins

Cat#	Target	Species	Conjugate	Expression host	Tag
10620-H03H-P	BCMA	Human	PE	HEK293	His & hFc
12238-HCCH-F	CD33	Human	FITC	HEK293	-
12238-HCCH-P	CD33	Human	PE	HEK293	-
10818-H08H-F	CD38	Human	FITC	HEK293	His
10818-H08H-P	CD38	Human	PE	HEK293	His

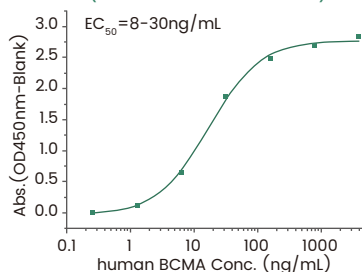
Sino Biological can offer a number of customer-customized labeling and recombinant protein expression services to meet specific goals, ranging from gene synthesis, vector construction to expression of tagged protein and purification.

## Biotin-labeled Target Proteins



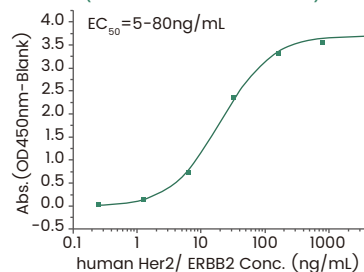
- Avi-tag biotinylated proteins feature with no interference in the protein's bioactivity
- High sensitivity and high specificity in detecting CAR expression positive rate
- Validated high activity and binding activity

Human BCMA recombinant protein  
(Cat#: 10620-H40H-B)



Immobilized Human BAFF/BlyS (hFc Tag)  
(Cat#: 10056-H01H) at 2 µg/mL (100 µL/well) can bind Human BCMA (His & AVI Tag), Biotinylated (Cat#: 10620-H40H-B), the EC<sub>50</sub> is 8-30 ng/mL.

Human Her2 recombinant protein  
(Cat#: 10004-H27H-B)



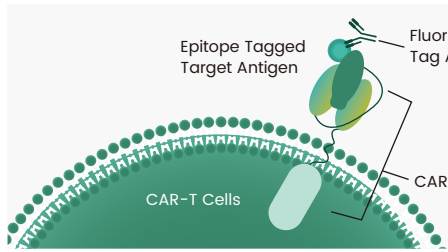
Immobilized Human Her2/ERBB2 (His & AVI Tag), Biotinylated (Cat#: 10004-H27H-B) at 2 µg/mL (100 µL/well) can bind Herceptin (Cat#: 68046-H002), the EC<sub>50</sub> is 5-80 ng/mL.

### List of More Biotinylated Proteins

Cat#	Target	Species	Expression host	Tag	Purity
10818-H27H-B	CD38	Human	HEK293	His & AVI	>95%
10673-H27H-B	NCAM1	Human	HEK293	His & AVI	>95%
29662-H27H-B	EGFR	Human	HEK293	His & AVI	>95%
10679-H27H-B	ULBP1	Human	HEK293	His & AVI	>95%
12143-H27H-B	ULBP2	Human	HEK293	His & AVI	>95%
13926-H27H-B	EPHA2	Human	HEK293	His & AVI	>95%
10692-H27H-B	c-MET	Human	HEK293	His & AVI	>95%
11027-H27H-B	CD5	Human	HEK293	His & AVI	>95%
11958-H41H-B	CD22	Human	HEK293	hFc & AVI	>90%
10350-H08H-B	IL13RA2	Human	HEK293	His	>95%
10012-H08H-B	VEGFR2	Human	HEK293	His	>95%
11077-H08H-B	CEACAM5	Human	HEK293	His	>95%
11880-H08H-B	CD19	Human	HEK293	His	>90%
12238-H08H-B	CD33	Human	HEK293	His	>90%
10777-H08H-B	CD30	Human	HEK293	His	>90%
29662-H08B-B	EGFR	Human	Baculovirus-Insect	His	>90%
15877-H07H-B	PSMA	Human	HEK293	His	>90%
10088-H08H-B	Glypican-3	Human	HEK293	His	>85%
10464-H07H-B	FAP	Human	HEK293	His	>85%
10084-H02H-B	PD-L1	Human	HEK293	hFc	>95%
10694-H02H-B	EpCAM	Human	HEK293	hFc	>90%
13128-H01H-B	Mesothelin	Human	HEK293	hFc	>85%
11007-H34E-B	CD20	Human	<i>E. coli</i>	TrxA	>85%
13968-HCCHI-B	ROR1	Human	HEK293	-	>95%
10004-HCCH-B	HER2	Human	HEK293	-	>95%

CAR-T Cell Quality Control

## Unconjugated Target Proteins



Sino Biological provides a wide collection of unconjugated CAR-T target proteins, which can be used to assess CAR expression in pair with fluorophore-labeled secondary antibodies.

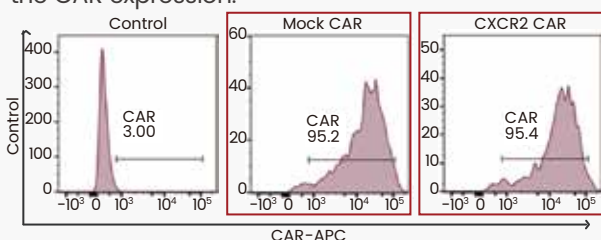
### List of More Unconjugated Proteins

Cat#	Protein	Species	Expression host	Tag	Purity	Bioactivity
11880-H08H	CD19	Human	HEK293	C-His	>90%	active
11958-h08h	CD22	Human	HEK293	C-His	>95%	active
11958-HNAH	CD22	Human	HEK293	Native	>95%	-
10777-H08H	CD30	Human	HEK293	C-His	>95%	active
10818-H32H	CD38	Human	HEK293	N-His & FLAG	>93%	-
10012-h02h1	VEGFR2	Human	HEK293	C-human IgG1-Fc	>95%	active
13926-h20b1	EPHA2	Human	Baculovirus-Insect	N-GST & His	>95%	active
11028-h08h	CD7	Human	HEK293	C-His	>95%	active
29662-h02b	EGFR	Human	Baculovirus-Insect	C-human IgG1-Fc	>95%	active
30025-hnah	CLDN18	Human	HEK293	Native	>90%	active
10088-h08h	Glypican-3	Human	HEK293	C-His	>87%	active
10692-H20B1	c-MET	Human	Baculovirus-Insect Cells	N-GST & His	>90%	active
10107-h08h	CA9	Human	HEK293	C-His	>95%	active
10084-h05h	PD-L1	Human	HEK293	C-mouse IgG1-Fc	>95%	active
10084-HNAH	PD-L1	Human	HEK293	Native	>95%	-
10121-H02H	ILIRAP	Human	HEK293	C-human IgG1-Fc	>95%	-
10518-H02H	CD123	Human	HEK293	C-human IgG1-Fc	>95%	-
10377-H08H	PD-1	Human	HEK293	C-His	>95%	active
10679-h03h	ULBP1	Human	HEK293	C-human IgG1-Fc & His	>95%	active
12143-H08H	ULBP2	Human	HEK293	C-His	>97%	-
15877-h07h	PSMA	Human	HEK293	N-His	>95%	active

## Successfully Supporting CAR-T Research

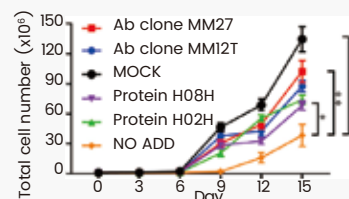
### Case 1

Title: CXCR2-modified CAR-T cells have enhanced trafficking ability that improves treatment of hepatocellular carcinoma (IF:6.688)  
 Journal: EUROPEAN JOURNAL OF IMMUNOLOGY  
 Author: Liu G., et al.  
 Citation product: GPC3 Protein (Cat#: 10088-H08H)  
 Application: FACS  
 Indirectly detection using GPC3 recombinant protein with His tag and its flow cytometry antibody to test the CAR expression.



### Case 2

Title: Blocking CD38-driven fratricide among T cells enables effective antitumor activity by CD38-specific chimeric antigen receptor T cells (IF:5.733)  
 Journal: The Journal of Genetics and Genomics  
 Author: Gao Z., et al.  
 Citation product: CD38 protein (Cat#: 10818-H08H and 10818-H02H) CD38 antibody (Cat#: 10818-MM12T and 10818-MM27)  
 Application: FACS, Blocking assay  
 Promote CAR-T cell proliferation by blocking CD38 recombinant protein and CAR scfv antibody.



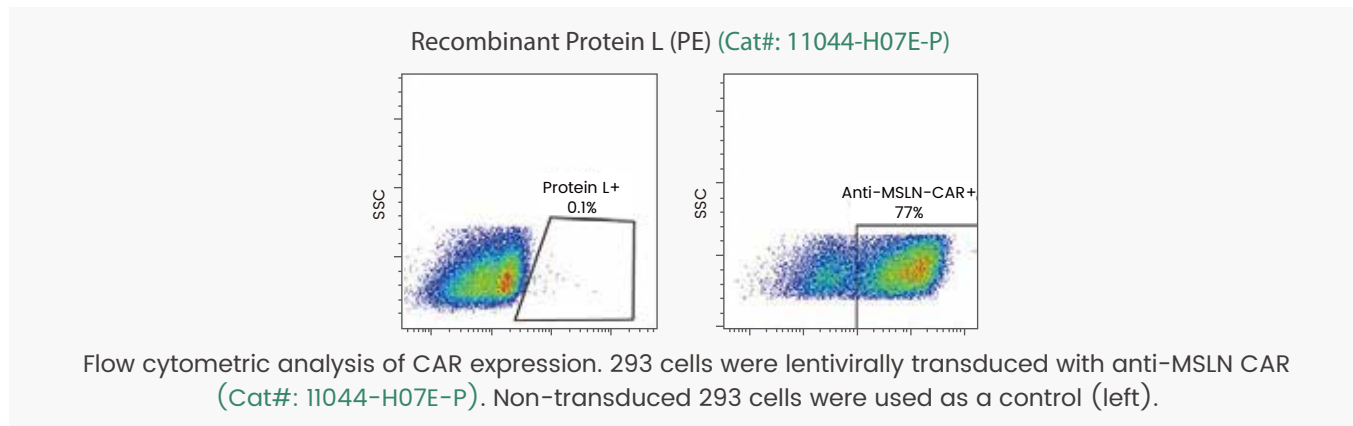
For full list, please visit : <https://www.sinobiological.com/category/car-t-cell-therapy-target-protein-elite>.



# Purity detection of CAR-T cells (Detection of Target Cells)

## Protein L

Protein L is a universal CAR detection reagent that can target light chain IgG-like fragments and be used for detection of CAR expression with different designs and different antigens. Protein L developed by Sino Biological has the features of high protein activity and high purity, and can be used for CAR-T analysis and detection.



## Anti-idiotype Antibody Production Services

Anti-idiotypic antibody (Anti-ID) recognizes scFv fragments on CAR structures with higher specificity and sensitivity, and has a lower non-specific background in fluorescence detection. Sino Biological provides a "one-stop" service from antigen preparation, anti-idiotype antibody development to assay establishment, facilitating the detection of CAR scFv.

### Service Highlights

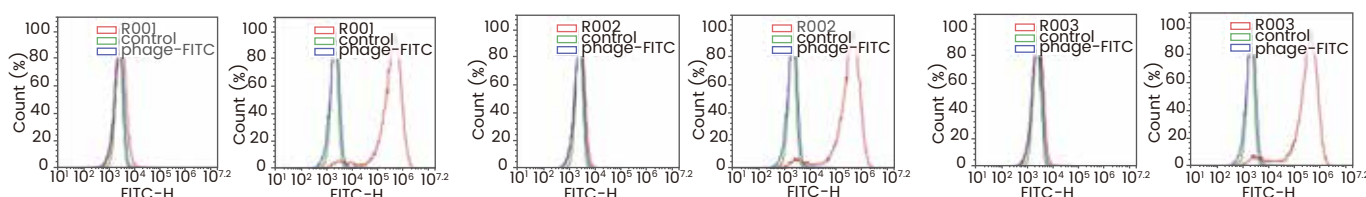
- Five Antibody Development Platforms: hybridoma, Beacon®, FACS B cell, phage display, and pAb technologies
- Multiple Purification Methods: Protein A, antigen affinity, total human IgG, Isotype IgG
- High Quality: high titer, high specificity, and low cross-reactivity with human IgG
- Rich Experience: serving clients all over the world with success rate >95%

### Anti-idiotypic Antibody Packages

Service	Deliverables	Time
Anti-ID Rabbit pAb Production Service	<ul style="list-style-type: none"> <li>• Purified antibodies</li> <li>• CoA</li> </ul>	2.5-4 months
Anti-ID Mouse mAb Production Service	<ul style="list-style-type: none"> <li>• Positive clones</li> <li>• Purified antibodies</li> <li>• CoA</li> </ul>	2-4 months
Anti-ID Rabbit mAb Production Service	<ul style="list-style-type: none"> <li>• Purified antibodies</li> <li>• Antibody H and L chain sequences</li> <li>• H and L chains in separate expression vectors</li> <li>• CoA</li> </ul>	4-6 months

## Featured Case Study of Anti-scFv mAb Generation

Immunizing rabbits with scFv, Sino Biological developed and screened multiple rabbit monoclonal antibody clones by phage display. These antibodies showed highly specific binding to cell lines overexpressing scFv in flow cytometry.



CAR-T Cell Quality Control

# CAR-T Cell Purity Testing (Impurities)

## ELISA Detection Kits

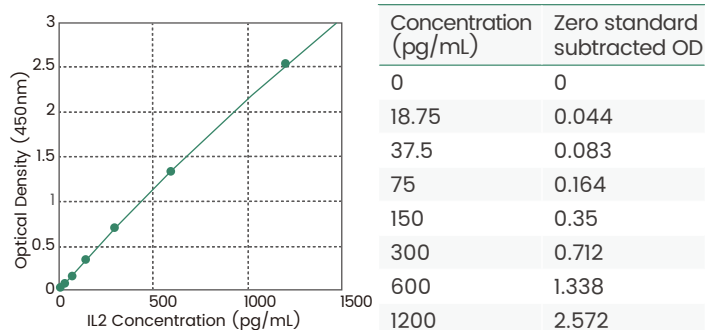
Process-related impurity detection refers to the impurities introduced in the process, such as residual proteases, transfection reagents, viral vectors, and cytokines, and should be investigated using appropriate methods.

During the production and preparation of CAR-T cells, some cytokine residues are present. At quality control step, the process residues such as cytokine residues should be tested. Sino Biological provides high quality IL-2 and IL-7 ELISA kits for cytokine residue detection.

### Human IL-2 Detection ELISA Kit

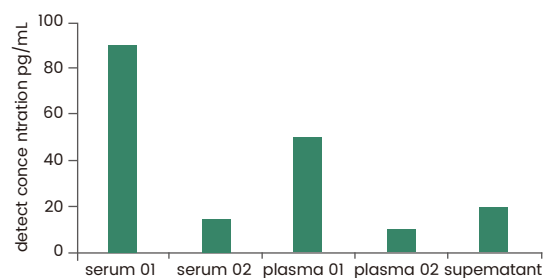
Cat#: KIT11848

#### • Standard curve



The standard curve of human Interleukin-2 (IL-2) quantitative ELISA Kit.

#### • Sensitivity



Detection of human serum, plasma and cell supernatant samples using Human IL-2 ELISA Kit.

#### • Precision

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	226	447	809	204	426	784
SD	3.73	9.63	24.73	24.19	20.52	25.15
CV(%)	1.60%	2.20%	3.10%	11.80%	4.80%	3.20%

It has been verified that the intra/inter-precision of human Interleukin-2 (IL-2) ELISA Kit are less than 10%.

#### • Recovery

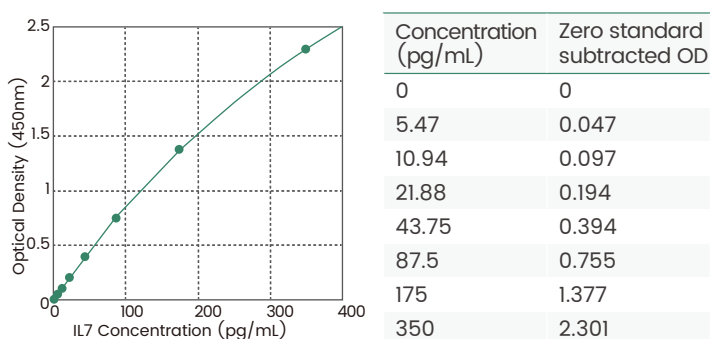
Sample	Average % Recovery	Range
serum (n=3)	76	74 -80%
supernatant (n=3)	99	90 -108%
plasma (n=3)	77	73 -82%

The average recovery of human IL-2 in serum, supernatant and urine samples were 76%, 99% and 77%, respectively, with assay recoveries ranging from 80% -120%.

### Human IL-7 Detection ELISA Kit

Cat#: KIT11821

#### • Standard curve



The standard curve of human Interleukin-7 (IL-7) ELISA Kit

#### • Precision

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	61	123	233	63	125	234
SD	1.3	2.98	9.02	0.71	2.43	6.99
CV(%)	2.10%	2.40%	3.90%	1.10%	1.90%	3.00%

It has been verified that the intra/inter-precision of human Interleukin-7 (IL-7) ELISA Kit are less than 10%.

## CAR-T Potency and Functionality Testing

The functional testing of CAR-T cells is an important step in the quality control of CAR-T cells, including cytokine release testing, in vitro killing ability, and intracellular signal transduction analysis. Sino Biological has developed high-quality cytokine ELISA kits, which can be used to detect the levels of representative cytokines to evaluate the killing activity and specificity of CAR-T cells on target cells. Furthermore, we also construct stable tumor cell lines, which can be used to evaluate the killing activity of CAR-T cells in vitro.

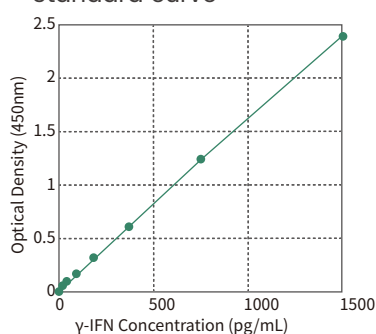
### ELISA Detection Kits

CAR-T cells are able to rapidly produce excessive amounts of cytokines when targeted to kill cancer cells, called cytokine release syndrome (CRS), they can activate more immune cells to respond to cancer cells, which cause tremendous immune effects and cause clinical manifestations such as fever, respiratory distress in patients, which are potentially fatal in severe cases. Therefore, it is particularly important to test the killing activity of CAR-T cells using cytokine ELISA kits.

According to the relevant guidelines, a complete non-clinical evaluation should include cytokine release assays to avoid or minimize the occurrence of potential safety events. Therefore, CAR-T cells should be closely monitored for a period of time after they are infused back into the patient to prevent safety risks. Sino Biological provides a variety of cytokine ELISA kits to satisfy the needs for detecting the level of cytokines during the development of CAR-T cell therapy products.

#### Human IFN gamma Detection ELISA Kit (Cat#: KIT11725A)

##### • Standard curve



Concentration (pg/mL)	Zero standard subtracted OD
0	0
23.4375	0.055
46.875	0.096
93.75	0.169
187.5	0.313
375	0.606
750	1.241
1500	2.391

##### • Recovery

Sample	Average % Recovery	Range
supernatant (n=3)	106	102-115%

The average recovery of human IFN gamma in supernatant were 106%, respectively, with assay recoveries ranging from 80% -120%.

The standard curve of human IFN gamma quantitative ELISA Kit.

##### • Precision

	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	179	387	844	240	491	942
SD	12.20	23.79	72.75	4.82	6.75	26.48
CV(%)	2.80%	6.10%	8.60%	2.00%	1.40%	2.80%

It has been verified that the intra/inter-precision of human IFN gamma are less than 10%.

##### • Linearity of dilution

Supernatant	
Dilution	Recovery of detected
1:02	100%
1:04	100%
1:08	95%
1:16	74%

The cell supernatant samples were diluted at 1:2, 1:4, 1:8 and 1:16, and the recovery of detected of IFN gamma protein was 74-100%.

### List of More Cytokine ELISA Kits

Molecule	Cat#	Species	Sample Type	Limit of Detection (pg/mL)	Linear Range (pg/mL)
IL-6	KIT10395A	Human	Serum, Cell culture supernatant	0.09	3.125-200
TNF- $\alpha$	KIT10602	Human	Cell culture supernatant	18.29	31.25-2000
IL-4	KIT11846	Human	Cell culture supernatant	2.54	10.94-700
GM-CSF/CSF2	KIT10015	Human	Cell culture supernatant	5.43	6.25-400
MCP-1/CCL2	KIT10134	Human	Serum, Urine	1.00	2.34-150
IL-8	KIT10098	Human	Cell culture supernatant	0.75	3.13-200
IL-10	KIT10947A	Human	Cell culture supernatant	7.25	15.63-1000
IL-12	KITCT011	Human	Cell culture supernatant	7.55	46.88-3000
IL-6R	KIT10398	Human	Serum, Urine	33.95	62.5-4000
IL-18	KIT10119	Human	Serum	11.83	31.25-2000
CRP	KIT11250	Human	Serum	21.78	54.69-3500
IL-5	KIT15673	Human	Cell culture supernatant	2.5	4.69-300

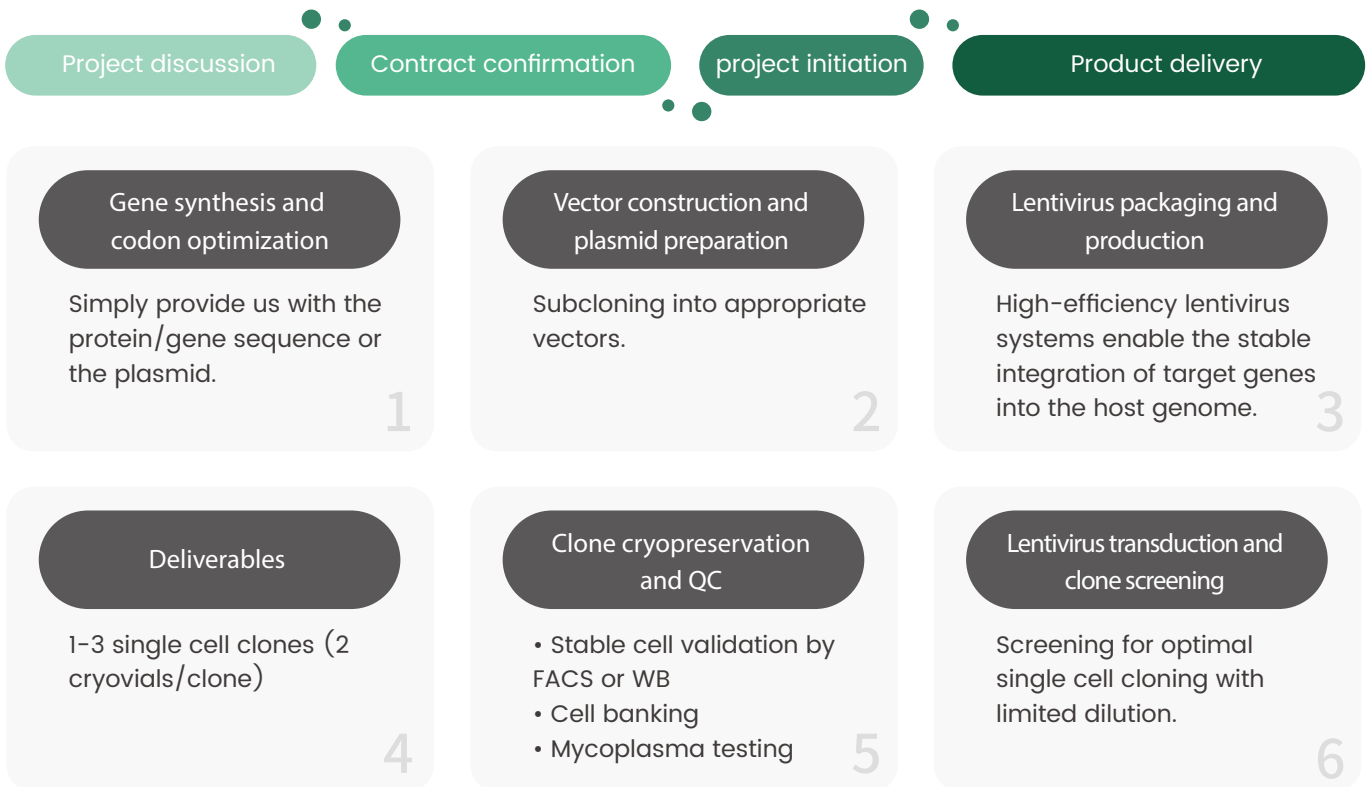
## Assay Cell Line Development Services

In the process of CAR-T cell quality control, T cell need to be verified by testing the efficacy of its killing effect in vitro.

With more than 15 years of rich experience in cell line development, Sino Biological provides a one-stop service to construct stable tumor cell lines, and deliver stable cell lines within 2-4 months, fully supporting the functional verification of CAR-T cells and other needs.

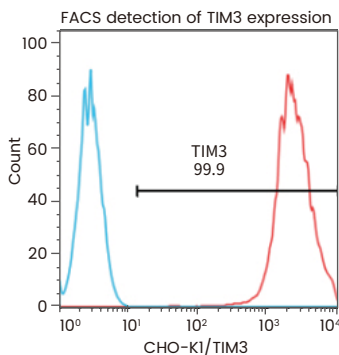


### Service Details



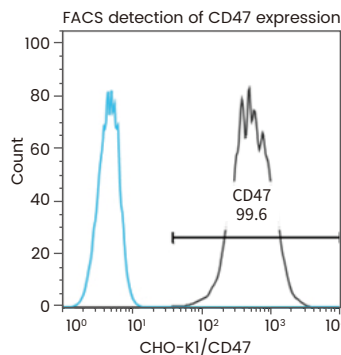
### Featured Case Studies

Single gene overexpression stable cell line



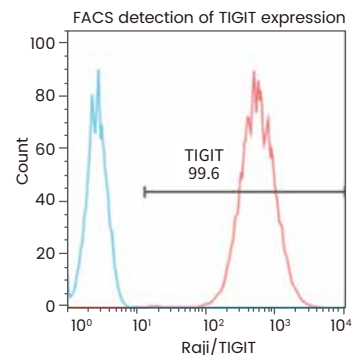
The expression of TIM3 was 99.9% detected by flow cytometry.

Multiple gene overexpression stable cell line



The expression of CD47 was 99.6% detected by flow cytometry.

Suspension cell line



The expression of TIGIT was 99.6% detected by flow cytometry.





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