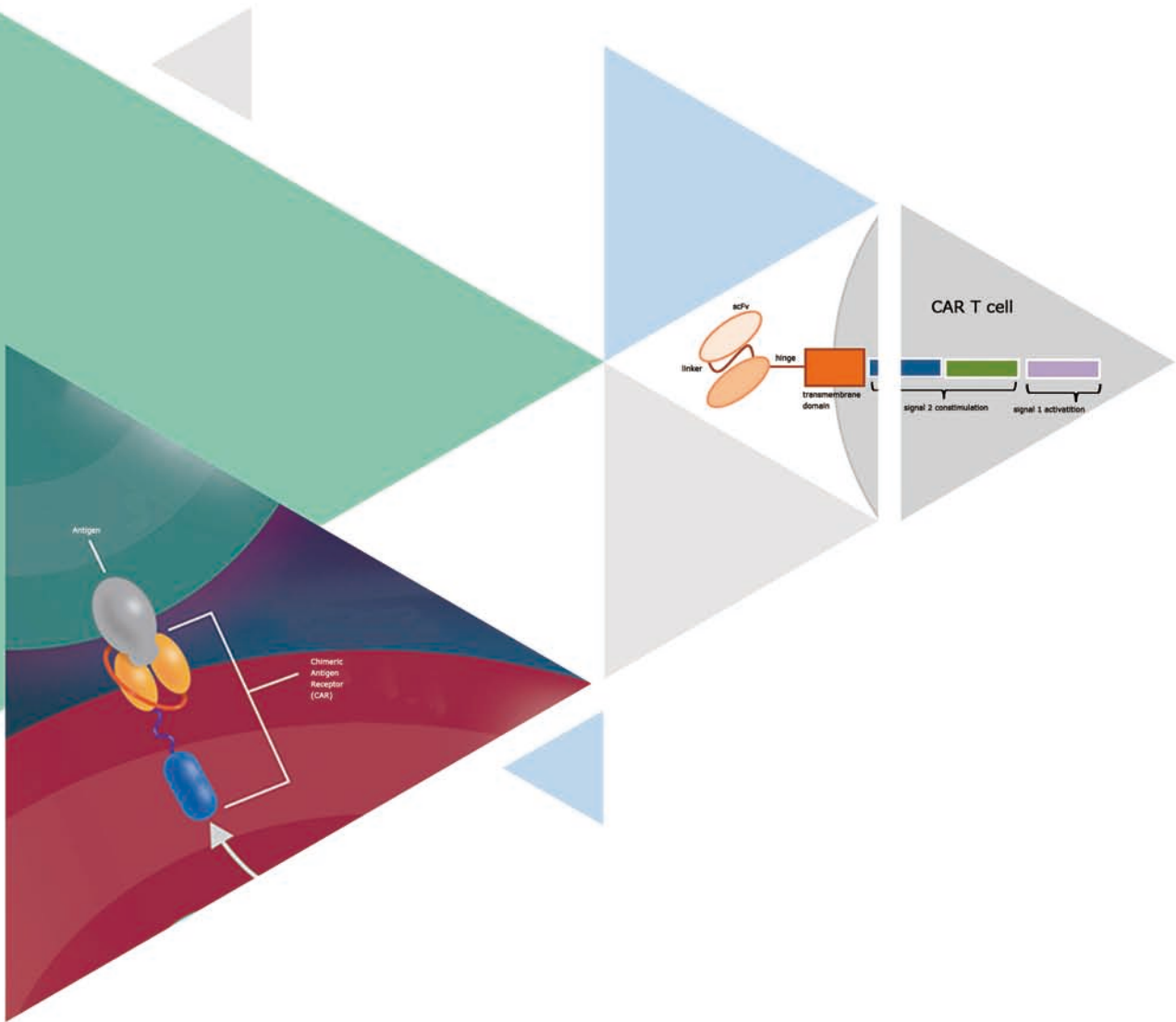


CAR-T Cell Therapy Research Tools



CAR-T Cell Therapy

CAR-T Introduction

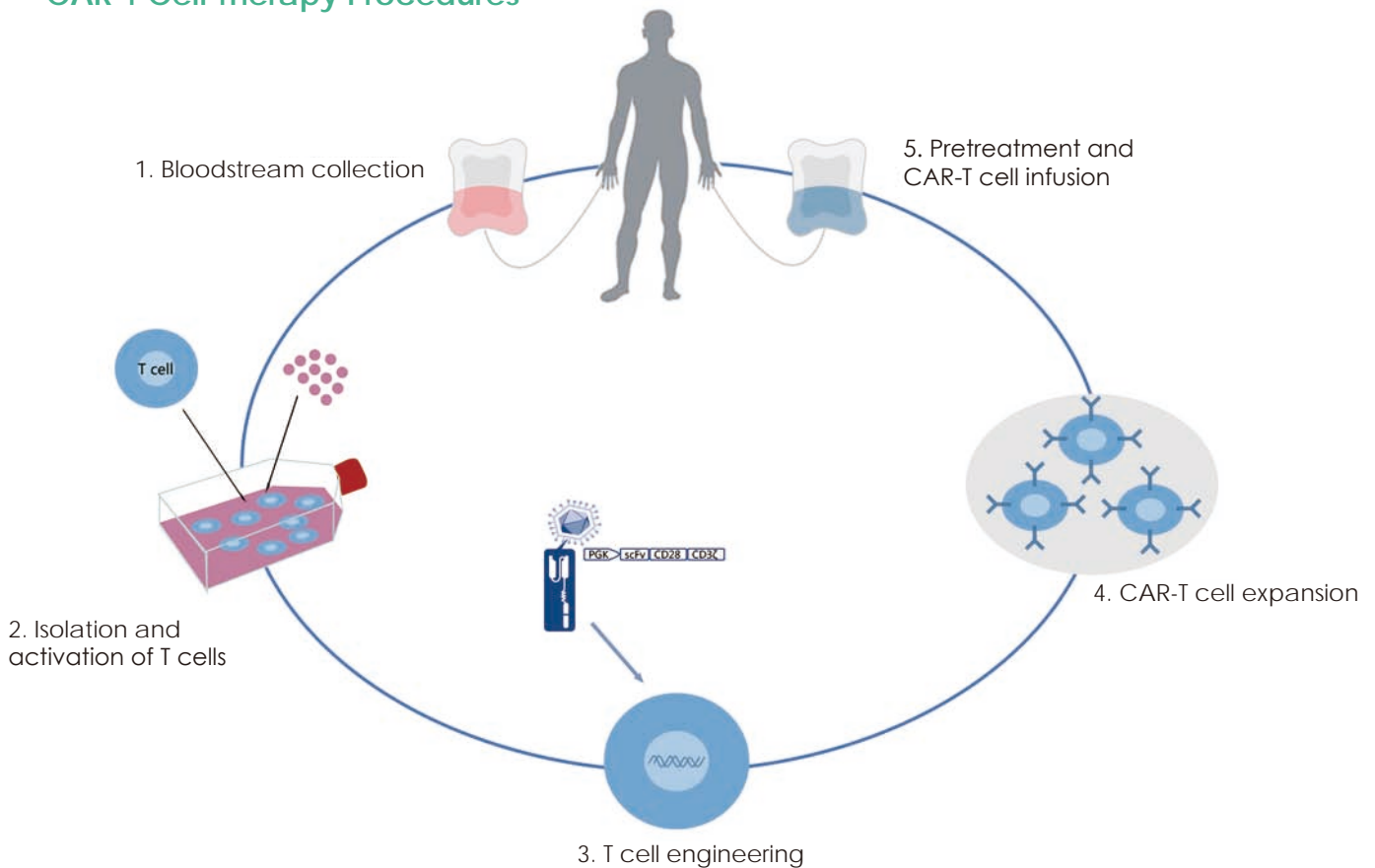
Chimeric Antigen Receptor T cell therapy, or CAR-T cell therapy, has been widely used in the field of cancer immunotherapy and clinical application.

The basic procedures for CAR-T cell therapy start with the collection and extraction of T cells from the tumor patient's peripheral blood. The T cells are then genetically engineered in vitro to make them express chimeric antigen receptors (CARs) that can recognize specific tumor-associated antigens and activate self-proliferation and cytotoxicity. Finally, CAR-T cells are expanded and reinfused into the patient, achieving the effect of recognizing and fighting the tumor.

CAR-T Events

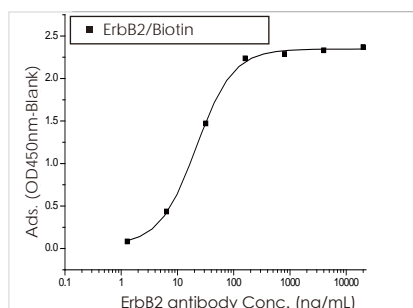
In 2012, Emily Whitehead became the first child in the world to receive an experimental CAR-T therapy. In August 2017, Novartis received FDA approval for a CAR-T cell therapy, Kymriah™. In October 2017, Kite Pharma received FDA approval for a CAR-T cell therapy, Yescarta™.

CAR-T Cell Therapy Procedures

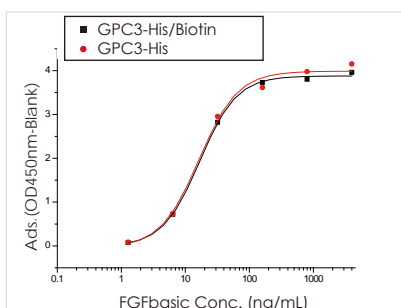


Solid Tumors Target Proteins

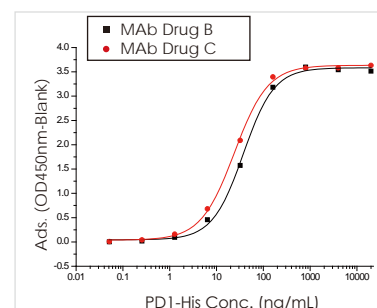
Solid tumor-associated antigens are expressed at low levels in normal tissues. Meanwhile, solid tumors have heterogeneity. Even within the same tumor region from the same patient, tumor cells might express different tumor-associated antigen. Therefore, choosing a target is a significant challenge for CAR-T cell therapy against solid tumors.



Binding of Biotinylated Human ErbB2 (Cat. No. 10004-H08H-B) to ErbB2 Antibody (Herceptin®) in a functional ELISA



Binding of Biotinylated Human GPC3 (Cat. No. 10777-H08H-B) and Unconjugated Human GPC3 Protein (Cat. No. 10777-H08H) to bFGF Protein in a functional ELISA



Binding of two PD-1 antibody drugs to Human PD-1 (Cat. No. 10377-H08H) in a functional ELISA

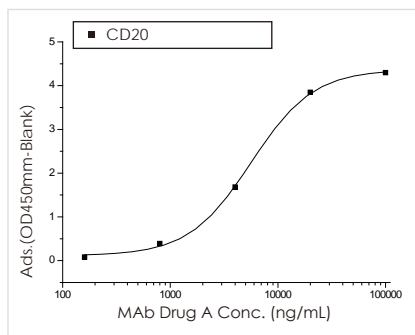
Table 1 Products List of Solid Tumors Targets

Molecule	Other Name	Host	Tumor Type
CEACAM5	CEA	Human cells	A variety of solid tumors
MET	c-MET	Human cells	Breast cancer
EGFR		Human cells/Insect cells	Colorectal cancer, Advanced EGFR positive solid tumors
EGFRvIII		Insect cells	Glioblastoma
EPCAM	CD326	Human cells	Hepatoma, Gastric cancer
EPHA2		Human cells	Malignant glioma
ERBB2	HER2	Human cells	A variety of solid tumors
GPC3	Glypican-3	Human cells	Hepatocellular carcinoma, Squamous cell lung cancer
MSLN	Mesothelin	Human cells	Mesothelioma, Pancreatic cancer, Ovarian cancer
Muc1	CA15-3	Human cells	Hepatocellular carcinoma, Non-small cell lung cancer, Pancreatic cancer, Breast cancer
PDCD1	PD-1	Human cells	Advanced lung cancer, Advanced liver cancer, Advanced gastric cancer
CD274	PD-L1	Human cells	Glioblastoma
KDR	VEGFR2	Human cells	Metastatic carcinoma, Metastatic melanoma, Renal cancer
IL13RA2		Human cells	Glioma
FOLH1	PSMA	Human cells	Prostate cancer
FAP		Human cells	Malignant pleural mesothelioma
CA9	CAIX	Human cells	Metastatic renal cell carcinoma
FOLR1	FR a	Human cells	Ovarian cancer
L1CAM	CD171	Human cells	Ovarian cancer, Neuroblastoma
ROR1		Human cells	Non-small cell lung cancer, Breast cancer, Leukemia

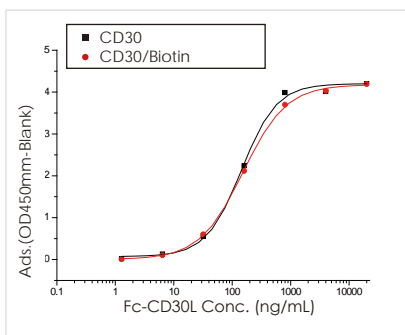
Sino Biological can provide all biotinylated proteins for targets listed above.

Hematologic Malignancies Target Proteins

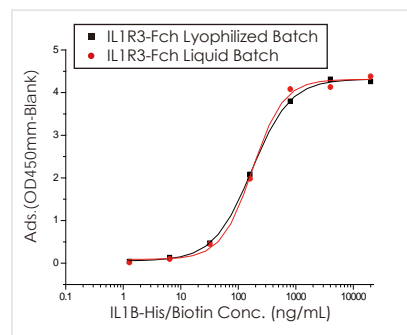
CD19 is the most widely used hematologic malignancy target in CAR-T cell therapy and good therapeutic effects have been achieved in clinical trials of hematological malignancies such as B-ALL, CLL, FL, DLBCL and MCL. In addition to CD19, molecules such as CD20, CD22, CD30, IL3RA are also hot targets for hematologic malignancies research, especially for CD19-negative hematologic malignancies. Sino Biological has a variety of hematologic malignancies target proteins, which can be used to detect CAR expression on T cells.



Binding of Human CD20 (Cat. No. 11007-H34E) to Anti-CD20 Mab Drug A in a functional ELISA



Binding of Biotinylated Human CD30 (Cat. No. 1077-H08H-B) and Unconjugated Human CD30 (Cat. No. 1077-H08H) to CD30L in a functional ELISA



Binding of human IL1R3 (Cat No: 10121-H03H) in lyophilized batch and liquid batch to IL1B in a functional ELISA

Table 2 Products List of Hematologic Malignancies Targets

Molecule	Other Name	Host	Tumor Type
TNFRSF17	BCMA	Human cells	Multiple myeloma
IL3RA	CD123	Human cells	Myeloid malignancies
SDC1	CD138	Human cells	Multiple myeloma
CD19		Human cells	Leukemia, Lymphoma, Multiple myeloma
MS4A1	CD20	E. coli	Leukemia, Lymphoma, Multiple myeloma
CD22	Siglec-2	Human cells	B-cell lymphoma
TNFRSF8	CD30	Human cells	Lymphoma
CD33	Siglec-3	Human cells	Myeloid malignancies
CD38		Human cells	Acute myeloid leukemia, Multiple myeloma
CD5	Leu-1	Human cells	T cell acute lymphoblastic lymphoma
NCAM1	CD56	Human cells	Acute myeloid leukemia
ULBP1	NKG2D ligand 1	Human cells	A variety of hematological malignancies
ULBP2	NKG2D ligand 2	Human cells	A variety of hematological malignancies
IL1RAP	IL1R3	Human cells	Chronic myeloid leukemia
ROR1		Human cells	Non-small cell lung cancer, Breast cancer, Leukemia

Sino Biological can provide all biotinylated proteins for targets listed above.

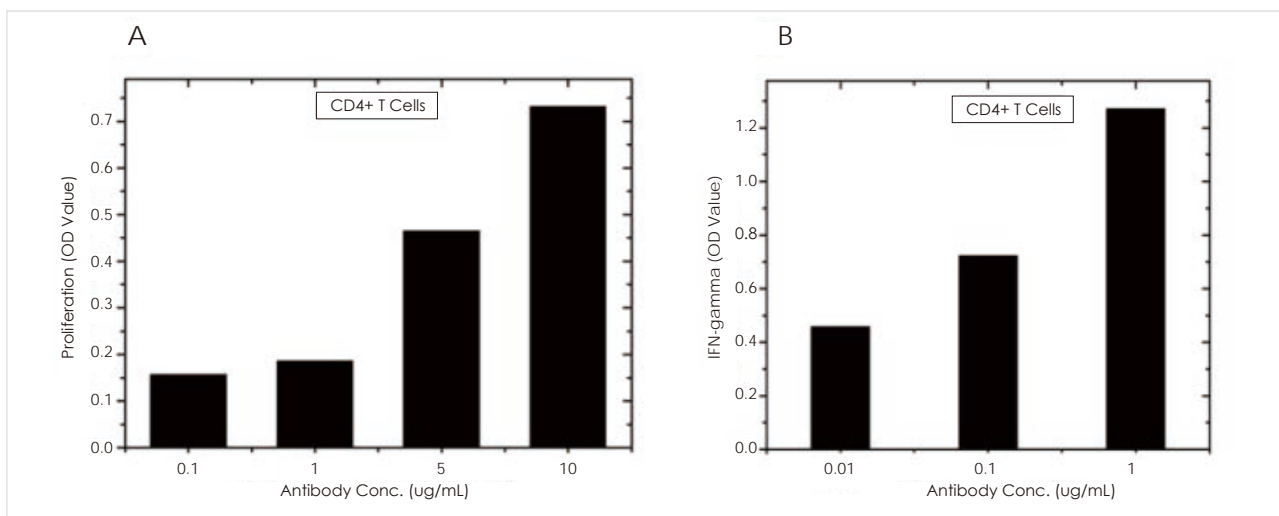
Table 3 Selected References Using Sino Biological Proteins in CAR-T Research

Molecule	Tag Type	Application	Magazine	Year
CD123	His tag	Detection of cell surface CAR expression (NK cell) by flow cytometry	Human gene therapy	2017
CEACAM5	His tag	Detection of cell surface CAR expression by flow cytometry	Molecular Therapy	2017
EGFR	His tag	Detection of cell surface CAR expression by flow cytometry	US 20140242701 A1	2014

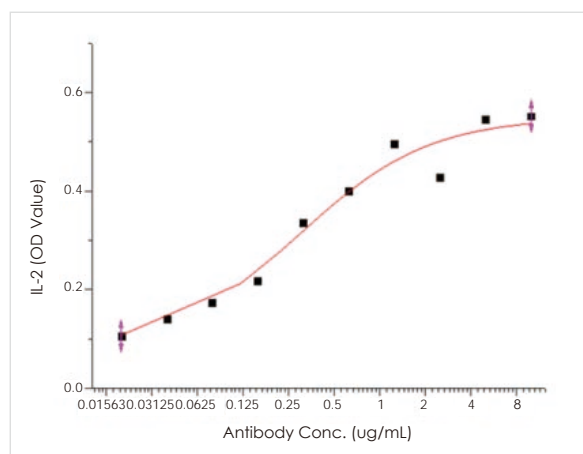
T cell Activation and Expansion

T cell Activation

Before T cell engineering, T cells which are collected and isolated from the patient's peripheral blood need to be activated. CD3, CD28 antibodies are commonly used for T cell activation in vitro.



Human CD3 antibody (Cat. No. 10977-H001) induces CD4+ T cell proliferation and IFN-gamma secretion.



Human CD28 antibody (Cat. No. 11524-H001) induces IL2 secretion of the CD4+ T cells. In the presence of 2 µg/mL of CD3 antibody, the IC50 of CD28 antibody is typically 0.2560 µg/mL.

Table 4 Sino Biological T Cell Activation Antibodies

Target	Purity (by HPLC)	Endotoxin (EU/mg)	Cat. No.
Human CD3 (humanized OKT3)	>95%	<0.5	10977-H001
Human CD28	>95%	<0.5	11524-H001

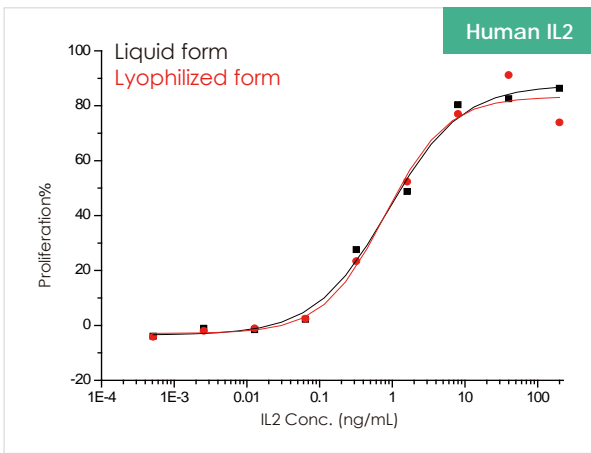
Sino Biological is capable of manufacturing GMP-level antibodies which will be on sale soon.

T cell Expansion

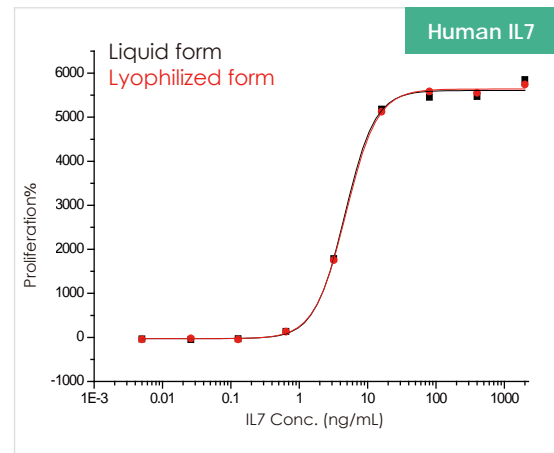
Sino Biological High-Quality Cytokines

- SEC-HPLC: ≥95%
- SDS-PAGE: ≥95%
- Endotoxin < 0.01 EU/μg
- Animal free
- Validated bioactivity
- Lot-to-lot consistency

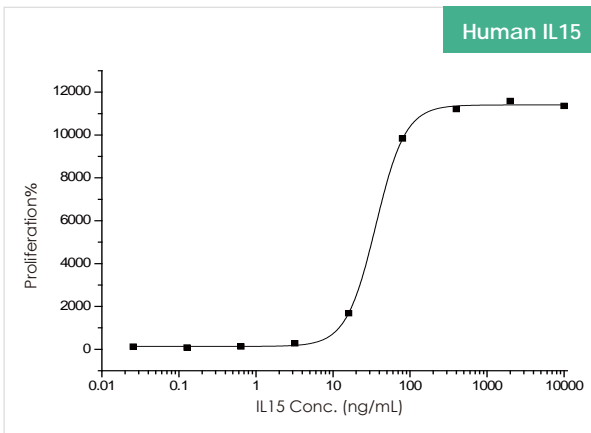
* Sino Biological is capable of manufacturing GMP-level cytokines which will be on sale soon.



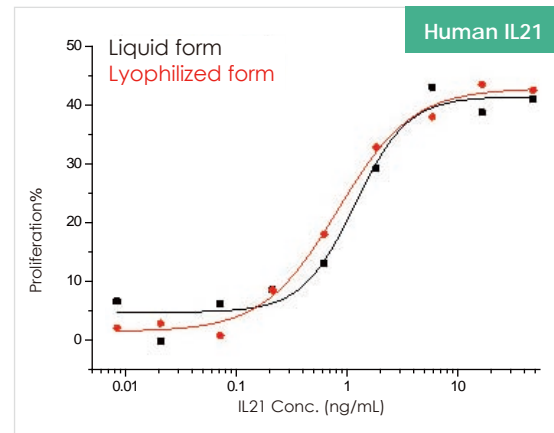
IL2 induces the expansion of CTLL2 mouse T lymphocytes.



IL7 induces the proliferation of human peripheral blood lymphocytes activated by CD3 antibody.



IL15 induces the proliferation of MO7e megakaryocyte lymphocytes.



IL21 induces IFN-gamma secretion in NK-92 human natural killer cells.

Table 5 High-Quality Cytokines

Cat. No.	Molecule	Cat. No.	Molecule
11848-HNAE	IL2	10584-HNAE	IL21
10015-HNAH	GMCSF	10602-HNAE	TNFα
10360-HNCE	IL15	10605-HNAE	EGF
10395-HNAE	IL6	11725-HNAS	IFN-gamma
11821-HNAE	IL7	11846-HNAE	IL4
CT011-H08H	IL12		

Cytokine ELISA Kits

Cytokines detection is one of the main ways to indicate CAR-T cell potency and it can also be used to study tumor immunosuppressive microenvironment. More than 90% ELISA Kits from SBI use recombinant proteins expressed in eukaryotic systems as controls. All ELISA Kits strictly follow eight QC standards including precision, recovery, range, the limit of detection (LOD), stability, natural sample test, cross-reactivity and interference to guarantee valid and reliable results.

Table 6 T Cell Related Cytokine ELISA Kits (Part of the List)

Molecule	Cat. No.	Sample	Limit of Detection (pg/mL)	Range (pg/mL)
TNF	KIT10602	PBMC supernatant	11.96	23.44-1500
IFNG	KIT11725A	PBMC supernatant	6.5	13.28-850
IL4	KIT11846	PBMC supernatant	2.54	10.94-700
IL6	KIT10395A	Serum, PBMC supernatant	0.69	3.13-200
IL10	KIT10947	PBMC supernatant	14.37	23.44-1500
CSF2	KIT10015	PBMC supernatant	2.04	7.81-500
CXCL9	KIT10888	Serum	11.96	23.44-1500
CCL2	KIT10134	Serum, Urine	1.9	3.91-250
IL13	KIT10369	PBMC supernatant	0.59	4.69-300
IL7	KIT11821	PBMC supernatant	1.89	6.25-400
IL17A	KIT12047	PBMC supernatant	0.58	1.88-120
CRP	KIT11250	Serum	12.8	46.88-3000

8 Stringent QC Test Indicators: Ensure high quality ELISA Kits

○ To evaluate the extent of the ELISA kit results close to actual values.

Recovery

A high or low recovery results in false positive or negative result affecting the accuracy of the measured value.

○ Make sure ELISA kit can detect natural samples and determine its applicable types of samples.

Natural sample test

If the types of natural sample are not confirmed, a false positive or negative result may be obtained.

○ To evaluate the specificity of the ELISA kit and determine the binding ability of other molecules to the paired antibodies.

Cross-reactivity

High cross-reactivity stands for low specificity.

○ To determine the effects of other molecules to the interaction of antigens with paired antibodies.

Interference

High interference stands for low accuracy of the measured value.

○ To ensure the stability of the ELISA kit for long-term storage.

Stability

Better stability of the ELISA kit means longer storage time.

○ To guarantee if similar results can be obtained when the same samples are repeatedly measured.

Precision

Poor precision of the kit will result in poor reproducibility when the same sample is repeatedly measured.

○ To determine the sensitivity of the ELISA kit.

The limit of detection (LOD)

Lower limit of detection stands for higher sensitivity.

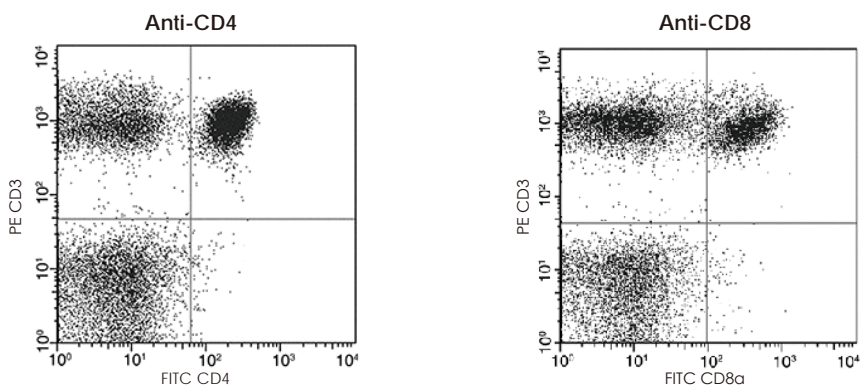
○ To assess the accuracy of the ELISA kit.

Range

High or low results of linear test will produce false positive or negative data.

T Cell Flow Cytometry (FACS) Antibodies

Flow cytometry is an important way to determine the potency and components of CAR-T cell, and the quality of FACS antibodies directly affects the success of experiments. Sino Biological develops a variety of T cell related FACS antibodies. Most of these antibodies were immunized by recombinant proteins expressed in eukaryotic systems, and they also have high specificity, high affinity and excellent S/N ratio, avoiding effectively the impact of FACS antibodies on the experiments.



Using CD4 antibody (Cat. No. 10400-MM08F) and CD8 antibody (Cat. No. 10980-MM28-F) to detect the expression of CD4 and CD8 on the surface of human PBMC by flow cytometry.

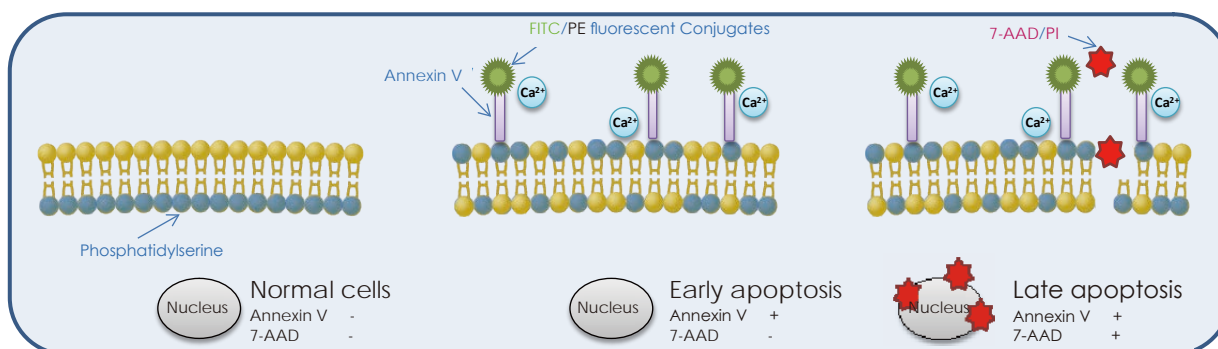
Table 7 T Cell FACS Antibodies (Part of the List)

Molecule	Antibody Name	Conjugate	Antibody Type	Target Species
CD3E	CD3e / CD3 epsilon Antibody	APC, PerCP, FITC, PE	Mouse MAb	Human
CD4	CD4 Antibody	APC, PerCP, FITC, PE	Mouse MAb	Human
CD8A	CD8/CD8 alpha/Leu-2 Antibody	APC, FITC	Mouse MAb	Human
IL-18R	IL18R1 Antibody	APC, FITC, PE	Mouse MAb	Human
CD26	DPP4 / CD26 Antibody	PE, APC, FITC	Mouse MAb	Human
TNF-α	TNF-alpha / TNFA / TNFSF2 Antibody	FITC	Mouse MAb	Human
TNF-α	TNF-alpha / TNFA / TNFSF2 Antibody	APC, FITC, PE	Rabbit MAb	Human
IFN-gamma	Interferon Gamma/IFN gamma/IFNG Antibody	APC, PerCP, FITC, PE	Rabbit MAb	Human
CD45RA	CD45RA Antibody	FITC, PE	Mouse MAb	Human
CD62L	CD62L / L-Selectin / SELL Antibody	APC, PerCP, PE	Mouse MAb	Human
CD38	CD38/ADPRC1 Antibody	APC, PerCP, FITC, PE	Mouse MAb	Human
CD25	IL2RA/IL-2RA/CD25 Antibody	FITC, PE	Mouse MAb	Human
CD25	IL2RA / IL-2RA / CD25 Antibody	APC, PerCP, FITC, PE	Rabbit MAb	Human
CD127	IL7R / IL-7R / CD127 Antibody	APC, PerCP, FITC, PE	Rabbit MAb	Human
CD73	CD73 / NT5E Antibody	FITC, PE	Mouse MAb	Human

Annexin V/7-AAD Apoptosis Detection Kits

- ▶ Produced in house
- ▶ Quality guarantee
- ▶ Bulk in stock
- ▶ Deliver in 24h

Detection Kit Principle

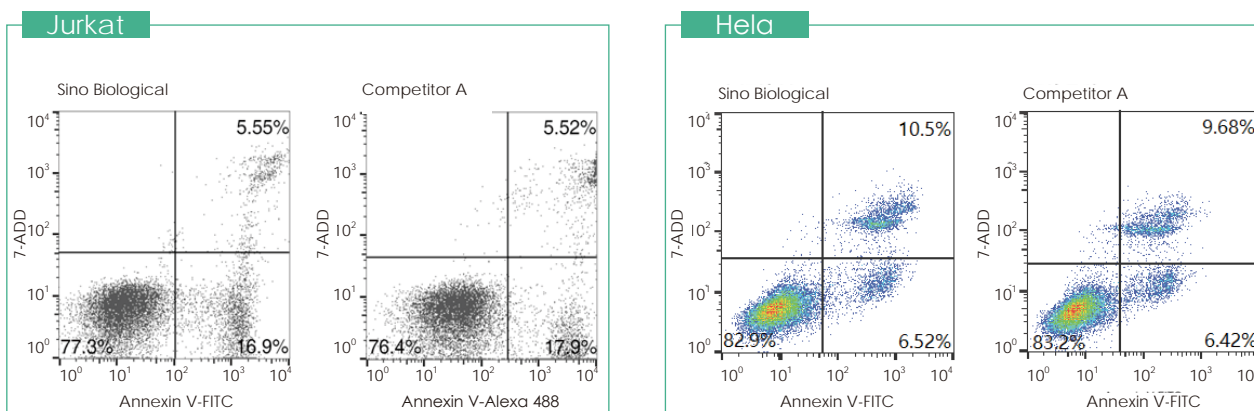


Annexin V combined with 7-AAD/PI staining method is easy to use, more time-saving, and capable of producing stable and reliable results. It's the **most ideal** method to detect cell apoptosis now.

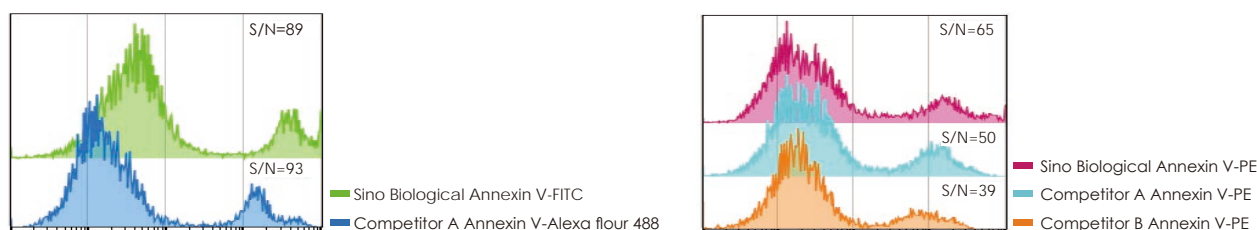
Tips: 7-AAD/PI staining method cannot distinguish between apoptosis and necrosis. It's recommended that more than two methods should be employed to comprehensively judge the apoptosis stage.

Reliable Sino Biological Apoptosis Detection Kits

Can accurately distinguish early and late apoptosis compared with leading competitor



Best S/N ratio, easy for setting gates to analyze, and more reliable results



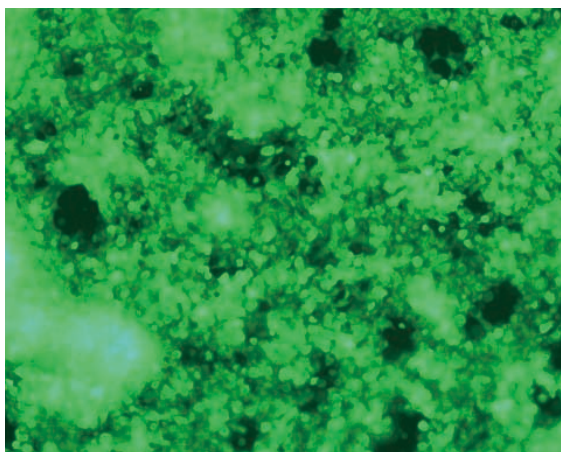
Sino Biological Annexin V/7-AAD Apoptosis Detection Kits List

Product Name	Cat. No.	Size
Annexin V-FITC/7-AAD Apoptosis Detection Kit	APK10448-F	20 Tests
Annexin V-FPE/7-AAD Apoptosis Detection Kit	APK10448-P	100 Tests

Lentiviral Vector Amplification

■ Transfection Reagent

Lentiviral vectors are widely used in CAR-T cell therapy for transducing CAR into T cells. Sino Biological's Sinofection® Transfection Reagent (Cat. No. STF02) can be applied to efficiently transfect lentiviral vectors, and the TCID50 of the obtained recombinant lentiviruses samples can reach over 10⁷.



Recombinant lentiviral vectors were transfected into adherent HEK293T cells by Sinofection® Transfection Reagent. After 72 hours Fluorescent images were taken to detect the expression intensity of EGFP protein.

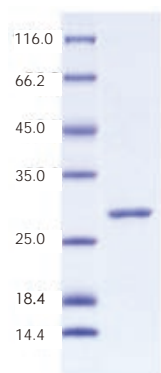


Recombinant lentiviral vectors (expressing EGFP protein) transfected by Sinofection® were retransfected into HEK293T cells. After 72 hours Fluorescent images were taken to detect the expression intensity of EGFP protein.

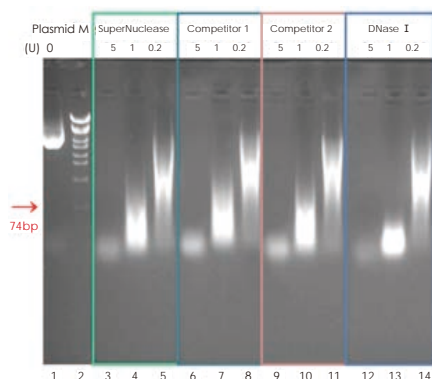
Supernuclease (Equivalent to Benzonase® Nuclease)

Supernuclease (Cat. No. SSNP01) is equivalent to Benzonase® Nuclease, can be used to remove DNA impurities in the process of lentiviral vector amplification. The HPLC purity of Supernuclease is over 99%. The DNA degradation rate is comparable with leading brands.

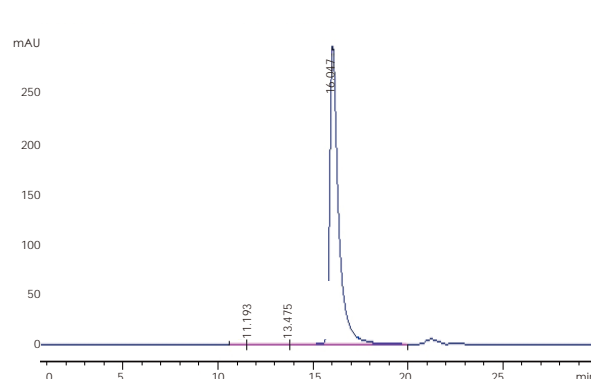
Purity by SDS-PAGE



DNA Degradation Rate



HPLC Detection Curve



Sino Biological Inc.

Established in 2007, Sino Biological is a global biotechnology company manufacturing high quality research reagents for basic, translational, and clinical research. Sino Biological laboratories consist of over 80,000 square meters, producing cDNAs, recombinant proteins, antibodies and antibody-based kits that are used to study cellular function and human diseases.

Sino Biological scientists are experienced in recombinant protein expression and purification from bacterial, yeast, insect and mammalian system. The scientists have also developed technologies to produce and purify a comprehensive collection of mouse monoclonal antibodies, rabbit polyclonal antibodies, and rabbit monoclonal antibodies. Sino Biological offers integrated service from gene cloning to protein expression, protein purification, and immunization for polyclonal or monoclonal antibody generation. We have provided CRO services to produce targets proteins, antibodies, and optimized ELISA tools to meet the needs of the biopharmaceutical industry, including the top 10 pharmaceutical companies.

Sino Biological product sales are supported through a network of distributors in North American, European and Asian markets. We are proud that our products are helping scientists to solve significant problems in life science research. Every product has to pass rigorous QC test standards to ensure consistent high quality. We validate our product application and guarantee its performance as specified. We are developing tools and technologies to provide the global scientific community with the best-in-class product to accelerate scientific discovery and innovation.

Advantages

■ Best Monoclonal Antibody Tools Worldwide

Maximum 200-Fold Enhanced ELISA sensitivity
Leading 2nd Generation Rabbit mAb Technology
No Products From Origin Entrusted Manufacturer

■ Centralized Protein & Antibody Expression Lab Worldwide

60+ Highly Centralized Cell Culture Bioreactors
6000+ Proteins Bulks in Stock
2000+ New Products Released Per Year
30-100 Grams Custom-Tailored mAb Production in Weeks

■ Leading Pre-Clinical CRO for Biologics Worldwide

One-stop Service from Discovery to Production
Record Speed from DNA to Purified mAbs
Serving Top-10 global Pharmaceutical/Biotech Companies