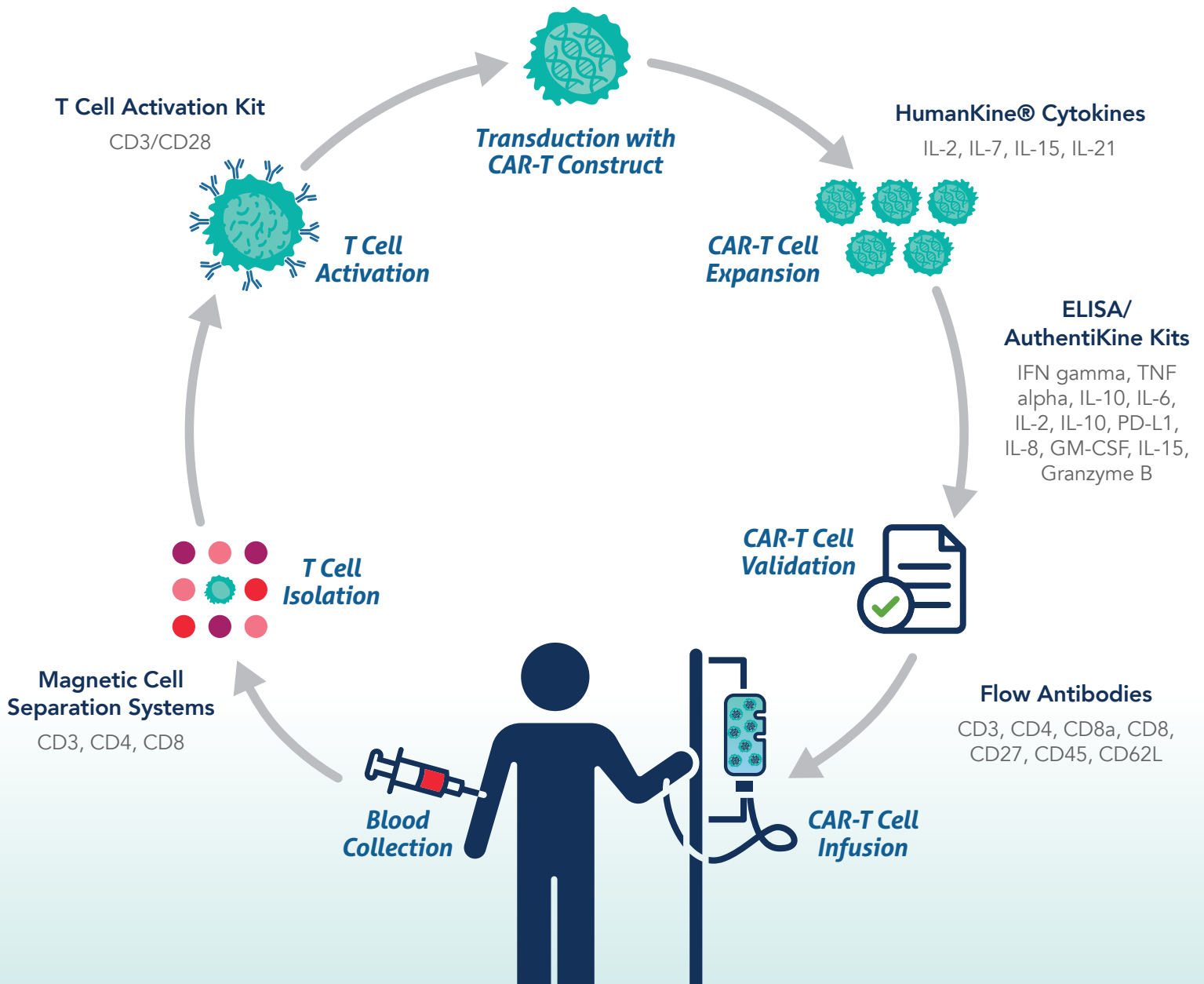# CAR-T Workflow

Proteintech now offers a complete workflow solution for customers working with Chimeric Antigen Receptor-T (CAR-T) cells. Because of the therapeutic nature of this research, the highest quality reagents are required to investigate and develop new immunotherapies. Proteintech's high quality, thoroughly validated products are all manufactured in-house and can be manufactured as GMP-grade to meet your quality requirements.

T Cell Activation Kit
CD3/CD28

Transduction with CAR-T Construct

T Cell Activation

HumanKine® Cytokines
IL-2, IL-7, IL-15, IL-21

CAR-T Cell Expansion

ELISA/ AuthentiKine Kits
IFN gamma, TNF alpha, IL-10, IL-6, IL-2, IL-10, PD-L1, IL-8, GM-CSF, IL-15, Granzyme B

CAR-T Cell Validation

T Cell Isolation

Magnetic Cell Separation Systems
CD3, CD4, CD8

Flow Antibodies
CD3, CD4, CD8a, CD8, CD27, CD45, CD62L

Blood Collection

CAR-T Cell Infusion

Cell Separation Products for T Cell Isolation and Activation

Proteintech offers several different magnetic bead-based cell separation systems for both positive and negative selection of desired T cell populations. A T cell activation kit can also be used to rapidly activate and expand isolated cell populations in culture.
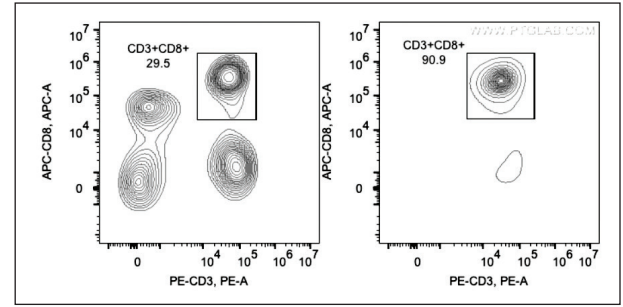
Product Name	Catalog No.	Method
CD3 Magnetic Beads	MS002	Positive Selection
CD3 Magnetic Beads Kit	KMS001	Positive Selection
CD4 Magnetic Beads	MS003	Positive Selection
CD4 Magnetic Beads Kit	KMS002	Positive Selection
CD8 Magnetic Beads	MS004	Positive Selection
CD8 Magnetic Beads Kit	KMS003	Positive Selection
Human CD4 Isolation Kit	KMS302	Negative Selection
Human CD3 Isolation Kit	KMS309	Negative Selection
Human CD8 Isolation Kit	KMS303	Negative Selection
Human CD3/CD28 T Cell Activation Beads Kit	KMS310	T Cell Activation

▲ **Human CD8 Isolation Kit (KMS303):** Following cell separation, cell suspension was stained with FITC-CD45, PE-CD3, and APC-CD8 antibodies. All CD45+ cells are gated in the analysis. Left panel: CD3+CD8+ cells before selection. Right panel: CD3+CD8+ cells after selection. Human CD8 selection kit is tested using PBMCs from three donors.
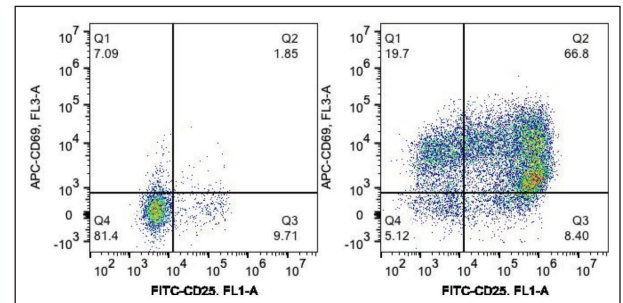
▲ **Human CD3/CD28 T Cell Activation Beads Kit (KMS310):** PBMCs were activated by Human CD3/CD28 T cell Activation Beads Kit for 3 days at a 1:1 bead to cell ratio. Unstimulated cells (left panel) and stimulated cells (right panel) were stained with fluorescence flow antibodies APC-CD69 and FITC-CD25. Total viable lymphocytes were gated. The activation effect of the kit was tested on human PBMCs from three different donors.

HumanKine® Cytokines for CAR-T Cell Expansion

Several cytokines are needed to maintain and expand CAR-T cells ex vivo. HumanKine® products, available both as RUO and GMP, can be used to culture these cells.

Protein	Catalog No.	Purity	Activity	Source	GMP-grade?
IL-2	HZ-1015	>95%	0.05-0.35 ng/mL	HEK293	Yes
IL-7	HZ-1281	>95%	0.2-1.4 ng/mL	HEK293	Yes
IL-15	HZ-1323	>95%	0.07-0.37 ng/mL	HEK293	Yes
IL-21	HZ-1319	>95%	0.25-1.25 ng/mL	HEK293	Yes

Learn more about our GMP policy at ptglab.com/products/gmp-grade-cytokines-growth-factors/

▲ **Superior Bioactivity of HumanKine® Recombinant IL-2 (HZ-1015):** Proteintech's HumanKine IL-2 (blue) was benchmarked with a leading competitor's product (red) on the same assay. The activity was determined by the dose-dependent stimulation of CTLIL-2L-2 (mouse cytotoxic T) cell proliferation. Humankine is nearly 40x more active than the competitor.

ELISA/AuthentiKine™ Kits to Determine CAR-T Cell Function

After expansion, CAR-T cells can be phenotypically characterized by the cytokines that they secrete. Proteintech offers several ELISA kits to assist in this detection process.

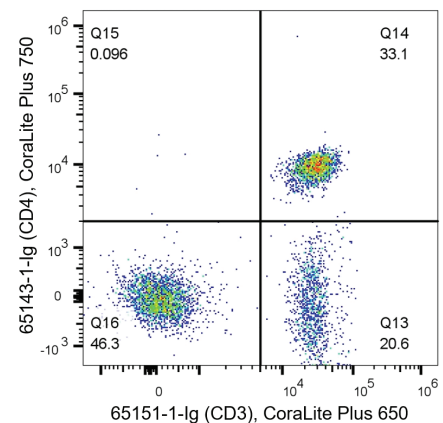| Target	Catalog No.	Species	Range	Sensitivity
GM-CSF	KE00003	Human	3.9-250 pg/mL	1.0 pg/mL
Granzyme B	KE00121	Human	15.6-1000 pg/mL	2.6 pg/mL
AuthentiKine IFN gamma	KE00146	Human	7.8-500 pg/mL	0.4 pg/mL
IL-2	KE00017	Human	15.6-1000 pg/mL	3.6 pg//mL
AuthentiKine IL-6	KE00139	Human	7.8-500 pg/mL	1.2 pg/mL
IL-8	KE00006	Human	15.6-1000 pg/mL	1.0 pg/mL
AuthentiKine IL-10	KE00170	Human	15.6-1000 pg/mL	2.3 pg/mL
IL-15	KE00102	Human	3.9-250 pg/mL	1.9 pg/mL
PD-L1	KE00074	Human	0.156-10 ng/mL	0.04 ng/mL
AuthentiKine TNF alpha	KE00154	Human	15.6-500 pg/mL	8.6 pg/mL

Anti-mouse ELISA kits are available at ptglab.com/products/elisa-kits/

FlexAble Antibody Labeling Kits for Customized Conjugations

In addition to the existing library of conjugated antibodies on the next page, Proteintech's FlexAble antibody labeling kits allow for the customized conjugation of any mouse IgG1 or rabbit IgG antibody to a dye of choice. This allows for simplified multiplexing when designing flow experiments to characterize mature CAR-T cells.

Conjugate	Catalog No.	Compatibility
CoraLite 488	KFA021	Mouse IgG1
	KFA001	Rabbit IgG
CoraLite Plus 550	KFA022	Mouse IgG1
	KFA002	Rabbit IgG
CoraLite Plus 650	KFA023	Mouse IgG1
	KFA003	Rabbit IgG
CoraLite Plus 750	KFA024	Mouse IgG1
	KFA004	Rabbit IgG

▲ **Flow Cytometry of PBMCs with CoraLite Plus 650 (KFA023) and CoraLite Plus 750 (KFA024):** PBMCs were stained with anti-CD3 (clone UCHT1, 65151-1-Ig) labeled with FlexAble CoraLite Plus Kit (KFA023) and anti-CD4 (clone RPA-T4, 65143-1-Ig) labeled with FlexAble CoraLite Plus 750 Kit (KFA024).

FlexAble kits have also been validated for additional applications including immunofluorescence and western blotting. Find out more at ptglab.com/products/flexable-antibody-labeling-kits/

Antibodies for Flow Cytometry

CAR-T cells can be distinguished by the expression of specific cell surface markers. Proteintech offers antibodies against many of these targets, conjugated in multiple formats for easy flow panel construction.

| Target	Clone	Unconjugated	FITC	CoraLite® 488	PE	APC	CoraLite® 647	CoraLite Plus 650
CD3	Hit3a	65112-1-Ig	FITC-65112	CL488-65112	PE-65112	APC-65112	CL647-65112	
	OKT3	65113-1-Ig	FITC-65133	CL488-65133	PE-65133	APC-65133	CL647-65133	
	SK7	65148-1-Ig	FITC-65148	CL488-65148	PE-65148	APC-65148	CL647-65148	
	UCHT1	65151-1-Ig	FITC-65151	CL488-65151	PE-65151	APC-65151	CL647-65151	
CD4	OKT4	65134-1-Ig	FITC-65134	CL488-65134	PE-65134	APC-65134	CL647-65134	CL650-65134
	RPA-T4	65143-1-Ig	FITC-65143	CL488-65143	PE-65143	APC-65143	CL647-65143	CL650-65143
	SK3	65147-1-Ig	FITC-65147	CL488-65147		APC-65147	CL647-65147	
CD8	SK1	65146-1-Ig	FITC-65146	CL488-65146	PE-65146	APC-65146	CL647-65146	
	UCHT4	65204-1-Ig	FITC-65204	CL488-65204			CL647-65204	CL650-65204
CD8a	Hit8a	65113-1-Ig	FITC-65113	CL488-65113		APC-65113	CL647-65113	
	OKT8	65135-1-Ig	FITC-65135	CL488-65135	PE-65135	APC-65135	CL647-65135	CL650-65135
	RPA-T8	65144-1-Ig	FITC-65144	CL488-65144	PE-65144	APC-65144	CL647-65144	
CD27	O323	65132-1-Ig	FITC-65132	CL488-65132	PE-65132	APC-65132	CL647-65132	
CD45RA	F8-11-13		FITC-65226					
	HI100	65108-1-Ig	FITC-65108	CL488-65108	PE-65108	APC-65108	CL647-65108	CL650-65108
CD45RO	UCHL1	65150-1-Ig	FITC-65150	CL488-65150	PE-65150	APC-65150	CL647-65150	
CD62L	DREG56	65167-1-Ig	FITC-65167					CL650-65167

Additional conjugated formats available at ptglab.com/products/flow-cytometry-antibodies/
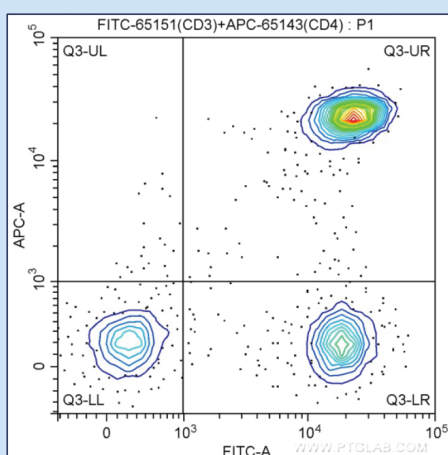
▲ **FITC Anti-Human CD3, clone UCHT1 (FITC-65151):** Human peripheral blood lymphocytes were stained with APC Anti-Human CD4 (clone RPA-T4) and FITC Anti-Human CD3 (clone UCHT1). Lymphocytes were gated for analysis. Cells were not fixed.
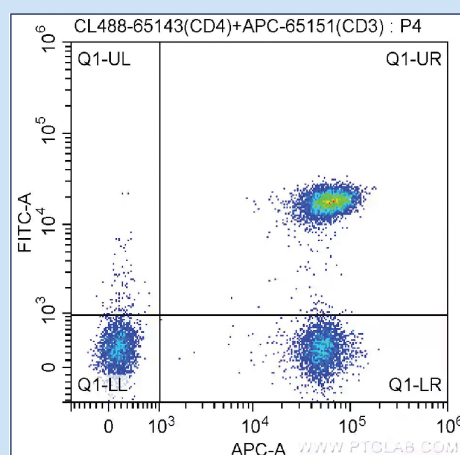
▲ **CoraLite® 488 Anti-Human CD4, clone RPA-T4 (CL488-65143):** Human peripheral blood lymphocytes were surface stained with APC Anti-Human CD3 (clone UCHT1) and CoraLite 488-conjugated Anti-Human CD4 (clone RPA-T4). Cells were not fixed
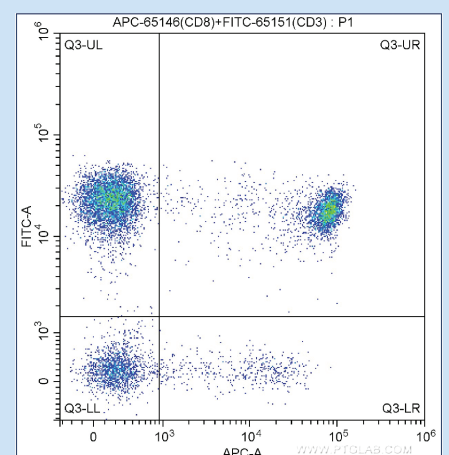
▲ **APC Anti-Human CD8, clone SK1 (APC-65146):** Human peripheral blood lymphocytes were surface stained with FITC Anti-Human CD3 (clone UCHT1) and APC Anti-Human CD8 (clone SK1). Lymphocytes were gated for analysis. Cells were not fixed.

Antibodies | ELISA kits | Proteins

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