

Materials List for

Generation and Functional Verification of Hypoxia-sensitive Chimeric Antigen Receptor-T Cells

Ying Xue^{*1}, Yunyu Mao^{*2}, Qibin Liao³, Chen Zhao⁴, Xiaoyan Zhang^{1,2,4}, Jianqing Xu^{1,2,4}

¹Institutes of Biomedical Sciences, Fudan University ²Clinical Center of Biotherapy at Zhongshan Hospital, Fudan University ³Department of Oncology and Bio-therapeutic Center, Shenzhen Third People's Hospital, Southern University of Science and Technology ⁴Shanghai Public Health Clinical Center, Fudan University

* These authors contributed equally

Corresponding Authors

Chen Zhao chen_zhao72@163.com
Xiaoyan Zhang zhangxiaoyan@fudan.edu.cn
Jianqing Xu xujianqing@fudan.edu.cn

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Materials

Name	Company	Catalog Number	Comments
1.5 mL Centrifuge tube	QSP	509-GRD-Q	Supernatants and cells collection Protocol Step 2,3,4
10% ExpressCast PAGE	NCM biotech	P2012	Immunoblotting Protocol Step 3
10x PBS	NCM biotech	20220812	Cell culture Protocol Step 4
10 mL pipette	Yueyibio	YB-25H	Pipetting Protocol Step 1
10xTRIS-Glycine-SDS electrophoresis buffer	Epizyme	3673020	Immunoblotting Protocol Step 3
15 mL Centrifuge tube	Thermo Scientific	339650	Supernatants and cells collection Protocol Step 1
25 cm ² EasYFlask	Thermo Scientific	156367	Cell culture Protocol Step 3,4
4x Protein SDS PAGE Loading Buffer	Takara	9173	Immunoblotting Protocol Step 3
6-well flat-bottom tissue culture plates	Thermo Scientific	140675	T Cells culture Protocol Step 1
96-well black flat-bottom tissue culture plates	Greiner	655090	Cytotoxicity assay Protocol Step 4
96-well ELISA plates	Corning	3590	ELISA Protocol Step 5
96-well plate shaker	QILINBEIER	MH-2	Shake Protocol Step 4
96-well U-bottom tissue culture plates	Thermo Scientific	268200	Supernatants collection Protocol Step 4,5
anti-FLAG antibody	Sigma	F1804-50UG	Immunoblotting Protocol Step 3
Carbinol	Sinopharm	10010061	Immunoblotting Protocol Step 3

Carbon dioxide incubator	Thermo Scientific	360	Cell culture Protocol Step 1,2,3,4
Cell counting plate	Hausser scientific	1492	Cell counting Protocol Step 1,3,4
CELLlection Pan Mouse IgG Kit	Thermo Scientific	11531D	Mouse IgG magnetic beads Protocol Step 1
Centrifuge	Thermo Scientific	75002432	Cell culture Protocol Step 1,3,4
Chemiluminescence gel imaging system	BIO-RAD	12003154	Immunoblotting Protocol Step 3
Cobalt chloride solution (0.5 M)	bioleaper	BR4000203	Hypoxic condition Protocol Step 2,3,4
DMEM	Corning	10-103-CV	Cell culture Protocol Step 4
Electronic balance	Sartorius	PRACTUM612-1CN	weigh Protocol Step 5
FBS	BI	04-001-1ACS	Cell culture Protocol Step 3,4
GAPDH Mouse mAb	ABclonal	AC002	Immunoblotting Protocol Step 3
Gel electrophoresis apparatus	BIO-RAD	1645070	Immunoblotting Protocol Step 3
GloMax Microplate Readers	Promega	GM3000	luciferase activity measurement Protocol Step 4
Goat anti-Mouse IgG (H+L)	Yeasen	P1126151	Immunoblotting Protocol Step 3
High speed microfreezing centrifuge	eppendorf	5810 R	Cell culture Protocol Step 1
Human IFN- γ ELISA Set	BD	555142	ELISA Protocol Step 5 Items: Recombinant Human IFN- γ Lyophilized Standard, Detection Antibody Biotin Anti-Human IFN- γ , Capture Antibody Purified Anti-Human IFN- γ , Enzyme Reagent Streptavidin-horseradish peroxidase conjugate (SAV-HRP)
Human IL-2 ELISA Set	BD	555190	ELISA Protocol Step 5 Items: Recombinant Human IL-2 Lyophilized Standard, Detection Antibody Biotin Anti-Human IL-2 , Capture Antibody Purified Anti-Human IL-2, Enzyme Reagent Streptavidin-horseradish peroxidase conjugate (SAV-HRP)
IL-15	R&D systems	P40933	T Cells culture Protocol Step 1
IL-21	Novoprotein	GMP-CC45	T Cells culture Protocol Step 1
IL-7	R&D systems	P13232	T Cells culture Protocol Step 1
Inverted microscope	Olympus	CKX41	Cell culture Protocol Step 1,3,4
Jurkat	ATCC	TIB-152	CAR-Jurkat construction Protocol Step 3
LSRFortessa	BD	LSRFortessa	Flow cytometry Protocol Step 2
Luciferase Assay System	Promega	E1501	luciferase reporter assay Protocol Step 4

			Items: Passive lysis buffer, firefly luciferase substrate
Microplate reader	BioTek	HTX	ELISA Protocol Step 5
mobile CO ₂ /O ₂ /N ₂ Incubator Chamber	China Innovation Instrument Co., Ltd.	Smartor118	Hypoxic condition Protocol Step 2, 3, 4
Mouse Anti-Hexa Histidine tag	Sigma	SAB2702218	Immunoblotting Protocol Step 3
NcmBlot Rapid Transfer Buffer	NCM biotech	WB4600	Immunoblotting
NcmECL Ultra	NCM biotech	P10300	Immunoblotting Protocol Step 3 Items: NcmECL Ultra Luminol/Enhancer Reagent (A) ,NcmECL Ultra Stabilized Peroxide Reagent (B)
NovoNectin -coated 48-well flat plates	Novoprotein	GMP-CH38	CAR-T cells construction Protocol Step 1
OPD (o-phenylenediamine dihydrochloride) tablet set	Sigma	P9187	Substrate Reagent Protocol Step 5 Items: OPD tablet (silver foil),urea hydrogen peroxide tablet (gold foil)
PE-conjugated anti-DYKDDDDK	Biolegend	637310	Flow cytometry Protocol Step 2
Protamine sulfate	Sigma	P3369-10G	Lentivirus infection Protocol Step 1
Protein Marker 10 Kda-250 KDa	Epizyme	WJ102	Immunoblotting Protocol Step 3
Purified NA/LE Mouse Anti-Human CD3	BD	566685	T Cells culture Protocol Step 1
Purified NA/LE Mouse Anti-Human CD28	BD	555725	T Cells culture Protocol Step 1
PVDF membrane	Millipore	168627	Immunoblotting Protocol Step 3
RPMI 1640	Corning	10-040-CVRC	Cell culture Protocol Step 3
Skim milk powder	Yeasen	S9129060	Immunoblotting Protocol Step 3
SKOV3-Luc	ATCC	HTB-77	Cytotoxicity assay Protocol Step 4
Trypsin-EDTA	NCM biotech	C125C1	Cell culture Protocol Step 4
Tween 20	Sinopharm	30189328	Immunoblotting Protocol Step 3
Water bath	keelrein	NB014467	Heating Protocol Step 1
X-VIVO 15	LONZA	04-418Q	Serum-free lymphocyte culture medium Protocol Step 1