



Premade TCR Lentivirus

Note: All products are for Research Use Only and CANNOT be used in the treatment or diagnosis of diseases.

Cat#	Product Name	Amounts
LVP1677	TCR-(MART-1) #1 (Clone DMF5) (No Marker)	200ul x (1x10 ⁸ IFU/ml) in PBS solution, pre-mixed with polybrene.
LVP1678	TCR-(MART-1) #1 (Clone DMF5) (Puro)	
LVP1679	TCR-(MART-1) #1 (Clone DMF5) (GFP-Puro)	
LVP1680	TCR-(MART-1)#2 (No Marker)	
LVP1681	TCR-(MART-1)#2 (Puro)	
LVP1682	TCR-(MART-1)#2 (GFP-Puro)	
LVP1683	TCR-(NY-ESO1) (Clone 1G4) (No Marker)	
LVP1684	TCR-(NY-ESO1) (Clone 1G4) (Puro)	
LVP1685	TCR-(NY-ESO1) (Clone 1G4) (GFP-Puro)	
LVP1686	TCR-(CD1d- α -GalCer) (Clone NKT15) (No Marker)	
LVP1687	TCR-(CD1d- α -GalCer) (Clone NKT15) (Puro)	
LVP1688	TCR-(CD1d- α -GalCer) (Clone NKT15) (GFP-Puro)	
LVP1689	TCR-(EB specific pMH), No selection	
LVP1690	TCR-(EB specific pMH) (Puro)	
LVP1691	TCR-(EB specific pMH) (GFP-Puro)	



LVP1692	TCR-(EB specific pMH)- CXCR3 (No selection)	
LVP1693	TCR-(EB specific pMH)- CXCR3 (Puro)	
LVP1694	TCR-(EB specific pMH)- CXCR3 (GFP-Puro)	

Storage: -80 °C, avoid repeat freeze/thaw cycles. Stable for 6 months.

1. Product Description:

1) About Lentivirus,

GenTarget's lentivector system utilizes Human Immunodeficiency Virus-1 (HIV) based plasmids for gene delivery. These lentivectors generate lentiviral particles (lentivirus) transducible into almost all mammalian cells, including stem cells, primary cells, and non-dividing cells in both in vivo and in vitro settings. Lentiviral particles stably integrate into the transduced cells' genome, ensuring long-term expression—a feature that makes them excellent gene transfer agents.

2) What is TCR?

T cell receptors (TCRs) are proteins found on T cells that bind to antigens on abnormal cells, cancer cells, cells from other organisms, and cells infected with a virus or another microorganism. TCR-T Cells, modified at their endogenous TCR gene, recognize tumor-specific antigens, activating the native T cell killing signal pathway.

3) What's difference between CARs and TCRs?

Unlike CAR-T cell therapy, which involves synthetic receptors, TCR cell therapy uses naturally occurring TCRs that recognize specific antigens. TCRs engage HLA-peptide complexes, requiring haplotype matching. TCRs recognize both cell surface and intracellular proteins, making them effective against solid tumors with fewer side effects compared to CAR-T therapy.

4) About CD3 ζ ,

CD3 ζ (T-cell receptor zeta) is expressed by T and NK cells, forming the TCR-CD3 complex. It transmits activation signals to T cells after antigen binding.

5) TCR Targets,

(1) MART-1 (Melanoma Antigen Recognized by T cells 1):



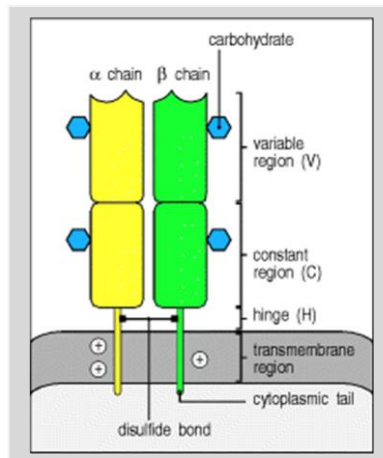
MART-1, also known as Melan-A, is a protein antigen found on the surface of melanocytes. The MART-1 antigen is a target in immunotherapy for patients with melanoma. TCRs targeting MART-1 recognize the antigen on melanoma cells, leading to T cell activation and cytotoxic T cell response to malignant melanoma cells.

- (2) NY-ESO1 (New York Esophageal Squamous Cell Carcinoma 1):
NY-ESO-1, short for New York Esophageal Squamous Cell Carcinoma 1, is a cancer-testis antigen, which means it is a protein normally expressed in embryonic testes but not in normal adult tissues. It has been found to be expressed in various cancer types, including melanoma, breast cancer, and ovarian cancer. The unique expression pattern of NY-ESO-1 makes it an attractive target for cancer immunotherapy.
- (3) CD1D (Cluster of Differentiation 1D):
CD1d is a non-classical major histocompatibility complex (MHC) class-I molecule. CD1d is a glycoprotein that presents lipid antigens to certain immune cells, such as natural killer T (NKT) cells.
- (4) α -Galactosylceramide (α -GalCer)
 α -GalCer is a glycolipid antigen that can bind to CD1d molecules. α -GalCer is a potent activator of invariant NKT (iNKT) cells. When α -GalCer is presented by CD1d, it activates NKT cells, a unique subset of T cells that bridge the innate and adaptive immune responses. α -GalCer has been utilized extensively due to its ability to induce potent activation of mouse and human iNKT cells.
- (5) CXCR3:
CXCR3 is a G protein-coupled receptor (GPCR) expressed on the surface of various immune cells, including T cells. The primary function of CXCR3 is to bind to specific chemokines, such as CXCL9, CXCL10, and CXCL11, which are often induced in response to inflammation. It plays a role in the recruitment of T cells to inflamed tissues, with enhanced antitumor immune responses.
By optimizing the homing of engineered T cells to tumor sites through the expression of CXCR3, it might be possible to improve the efficacy of TCR cell therapies in the context of cancer immunotherapy.

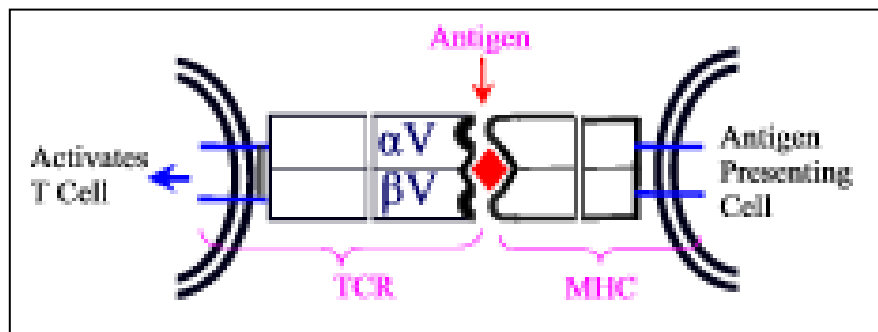


2. Gentarget's TCR Lentivirus:

TCR consists of T cell receptor α (TCR α) and β (TCR β) chains, linked by a disulfide bond. These α : β heterodimers are very similar in structure to the **Fab** fragment of an immunoglobulin molecule, and they account for antigen recognition by most T cells. The following scheme demonstrates the TCR structure.



MHC molecules are cell-surface glycoproteins with a peptide-binding groove that can bind a wide variety of different peptides. Every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities. TCR engages MHC-peptide complexes (Type I in CD8 cells and Type II in CD4 cells) in T cell activation, meaning the MHC needs to be matched between TCR-T cells and the target cancer cells (between T cell and the patient's haplotype). A given T cell is specific for a particular peptide bound to a particular MHC molecule. The scheme below illustrates the activation of T-cells.



Gentarget Inc constructs a set of TCR Lentivirus products. Each TCR recognize a specific cancer antigen. Upon transduced with TCR Lentivirus, the T cells will be



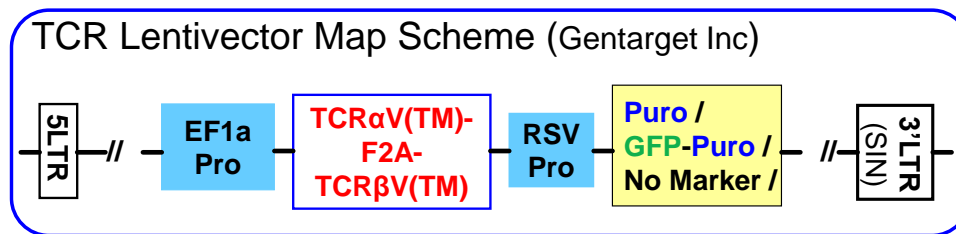
genetically modified and activated as cytotoxic T cell that kill target cells containing the specific antigen.

(1) TCR Lentivector structure:

The TCR Lentivector expresses TCR α V and TCR β V under the enhanced EF1a promoter, with a selection marker under the RSV promoter. Each lentivector includes a selection, either Puromycin, or GFP-Puromycin dual-selection, or no selection as desired.

TCR cassette components are co-expressed but not fused (mediated by F2A element), allowing TCR structure formation. The TCR cassette express the antigen-specific variable (V) region of both TCR α and TCR β with its transmembrane (TM) segment. When desired, a co-stimulator is also co-expressed mediated by T2A element.

The TCR cassette is "TCR α V(TM)-F2A-TCR β V(TM)" as demonstrated in the scheme below.



The "target-specific TCR α V and TCR β V sequences" are derived from the verified clones according to published literatures.

(2) Lentivirus details:

VSV-G pseudotyped, replication-incompetent CAR-T Lentivirus is provided in PBS solution at a titer of 1×10^8 IFU/ml, 200ul/vial. For more details, please see [FAQs for pre-made lentiviral particles \(.pdf\)](#).

3. Key features of Gentarget's TCR Lentivirus:

- (1) Great tool to generate TCR-T cells killing cancer cells.
- (2) High-titer lentivirus for high transduction rates in T cells.
- (3) Strong promoter strength in T cells using the enhanced EF1a promoter.
- (4) Easy transduction verification by GFP fluorescent signal.



- (5) Transduced cells can be sorted via a fluorescent signal or selected for antibiotic resistance.
- (6) Ready-to-use: Add 50 to 100 μ l to your cell culture and leave for 48 to 72 hours.

4. Transduction Protocol in T Cells (lymphocyte):

Note: Pre-made lentivirus is ready to use.

- (1) Seed 0.5 ml of T lymphocyte cells at a concentration of 1 million cells/ml into a 24-well plate at the time of lentivirus transduction.
- (2) Thaw the Lentivirus, add 50 μ l to 100 μ l of CAR-T lentivirus into each well, and return cells to 37°C.
- (3) At 48 to 72 hours post-transduction, transfer cells for enrichment and expansion.
- (4) Check transduction rate by fluorescence microscopy or flow cytometry after 1 week.

Filter wavelength settings:

GFP filter: Ex450-490, Em525.

If you do not use all of the lentivirus at one time, you may re-freeze the virus at -80 °C for future use; lentivirus titer will decrease by ~10% for each freeze/thaw cycle.

5. TCR assay workflow (for reference only):

- (1) Culture:
Culture desired T cells, isolated from patient's blood samples, and apply positive enrichment and negative removal.
For example, use micro-Beads (CD8 kit for T, or CD56 kit for NK) to isolate the desired T cell sub-group. The T cells has the CD3 and CD8 positive. The NK cells are CD56 positive.
- (2) Stimulation/Activation:
 - 1) Select the desired CAR or TCR Lentivirus;
 - 2) transduce cells (see Transduction Protocol above);
 - 3) Expansion of the Engineered cells;
Culture the T cells with desired cytokine for activation (For example, add IL2, IL7 for T cells, IL15 for NK cells).



(3) Verification:

Conduct target killing cell assays or measure secreted cytokines.

Note: Results may vary due to the assay's research nature. Gentarget Inc cannot guarantee your assay's results. Gentarget Inc only can guarantee the lentivirus' quality (the virus titer and the CAR/TCR sequence accuracy).

6. Safety Precaution:

Gentarget lentiviral particles, featuring advanced safety features, are replication-incompetent. Use caution in a Bio-safety II cabinet, wearing gloves. Refer to CDC and NIH guidelines for safety.

7. References:

1. [The Emerging World of TCR-T Cell Trials Against Cancer: A Systematic Review; Technology in Cancer Research & Treatment February 24, 2019](#)
2. [The molecular bases of \$\alpha/\beta\$ T cell - mediated antigen recognition. J. Exp. Med. 2014 Vol. 211 No. 13 2599–2615](#)
3. [Development of allogeneic HSC-engineered iNKT cells for off-the-shelf cancer immunotherapy. Li et al., 2021, Cell Reports Medicine 2, 100449](#)
4. [A structural basis for selection and crossspecies reactivity of the semi-invariant NKT cell receptor in CD1d/glycolipid recognition, JEM, Vol. 203, No. 3, March 20, 2006 661–673](#)
5. NIH Guidelines for [Bio-safety Considerations for Research with Lentiviral Vectors](#). (Link).
6. [CDC guidelines for Lab Bio-safety levels \(Link\)](#).

8. Warranty:

This product is for research use only and is warranted to meet its described quality when used according to instructions. GenTarget disclaims any implied warranty for a particular application. In no event shall GenTarget be liable for incidental or consequential damages. GenTarget's sole remedy for breach of this warranty is to replace the products, at its option.

9. Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Reporter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.



Product Category	Product Description (please click into each category's page)
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGF β ; TCRs : MART-1/ NY-ESO1/ CD1d- α -GalCer/ TR α V3-F2A-TR β V5-6;
CRISPR Gene Editing	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research
Epigenomic: CRISPRi and CRISPRa	" dCas9-Protein " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
Infectious Antigens	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verified and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
Anti-miRNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.



Product Category	Product Description (please click into each category's page)
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla, Red-Luc and more , with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwitch	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF



Product Category	Product Description (please click into each category's page)
	inducible system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FGF4) factors with different fluorescent and antibiotic markers
LacZ expression	Express different full length β-galactosidase (lacZ) with different selection markers
Negative control lentiviruses	Premade negative control lentivirus with different markers : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.