GenTarget Inc

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Premade CAR-T Lentivirus

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

Cat#	Product Name	Amounts
LVP1641	Anti-**CD19_CD20**-ScFv-4-1BB-CD3ζ (No Select)	200ul x ($1x10^8$ IFU/ml) in PBS solution, pre-mixed with polybrene.
LVP1642	Anti-**CD19_CD20**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1643	Anti-**CD19_CD20**-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1644	Anti-**CD19_CD20**-ScFv- CD28-CD3ζ (No Select)	
LVP1645	Anti-**CD19_CD20**-ScFv- CD28-CD3ζ (Puro)	
LVP1646	Anti-**CD19_CD20**-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1647	Anti-**BCMA**-ScFv-4-1BB-CD3ζ (No Select)	
LVP1648	Anti-**BCMA**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1649	Anti-**BCMA**-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1650	Anti-**BCMA**-ScFv- CD28-CD3ζ (No Select)	
LVP1651	Anti-**BCMA**-ScFv- CD28-CD3ζ (Puro)	
LVP1652	Anti-**BCMA**-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1653	Anti-**h HER2**-ScFv-4-1BB-CD3ζ (No Select)	
LVP1654	Anti-**h HER2**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1655	Anti-**h HER2**-ScFv-4-1BB-CD3ζ (GFP-Puro)	

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LVP1656	Anti-**h HER2**-ScFv- CD28-CD3ζ (No Select)	
LVP1657	Anti-**h HER2**-ScFv- CD28-CD3ζ (Puro)	
LVP1658	Anti-**h HER2**-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1659	Anti-**h CD22**-ScFv-4-1BB-CD3ζ (No Select)	
LVP1660	Anti-**h CD22**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1661	Anti-**h CD22**-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1662	Anti-**h CD22**-ScFv- CD28-CD3ζ (No Select)	
LVP1663	Anti-**h CD22**-ScFv- CD28-CD3ζ (Puro)	
LVP1664	Anti-**h CD22**-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1665	Anti-**h HLA-A2**-ScFv-4-1BB-CD3ζ (No Select)	
LVP1666	Anti-**h HLA-A2**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1667	Anti-**h HLA-A2**-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1668	Anti-**h HLA-A2**-ScFv- CD28-CD3ζ (No Select)	
LVP1669	Anti-**h HLA-A2**-ScFv- CD28-CD3ζ (Puro)	
LVP1670	Anti-**h HLA-A2**-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1671	Anti-**h TGFb**-ScFv-4-1BB-CD3ζ (No Select)	
LVP1672	Anti-**h TGFb**-ScFv-4-1BB-CD3ζ (Puro)	
LVP1673	Anti-**h TGFb**-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1674	Anti-**h TGFb**-ScFv- CD28-CD3ζ (No Select)	

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LVP1675	Anti-**h TGFb**-ScFv- CD28-CD3ζ (Puro)	
LVP1676	Anti-**h TGFb**-ScFv- CD28-CD3ζ (GFP-Puro)	
CAR-ctr5	CAR negative control: 4-1BB-CD3ζ (No Select)	
CAR-ctr1	CAR negative control: 4-1BB-CD3ζ (Puro)	
CAR-ctr7	CAR negative control: 4-1BB-CD3ζ (GFP-Puro)	
CAR-ctr6	CAR negative control: CD28-CD3ζ (No Select)	
CAR-ctr3	CAR negative control: CD28-CD3ζ (Puro)	
CAR-ctr8	CAR negative control: CD28-CD3ζ (GFP-Puro)	

Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for 6 months.

1. Product Description:

1) **About Lentivirus**,
GenTarget's lentivector system employs 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 CAR-T**,
CARs (Chimeric Antigen Receptors) modify T cells, imparting a unique surface structure comprising antigen-binding and T cell activation domains.

The antigen-binding moieties typically involve single-chain variable fragments (scFv) derived from antibodies, such as those targeting a tumor surface antigen, or may include Fab segments or natural ligands binding to their cognate receptors. The activation segment comprises a chimeric molecule activation domain (1st generation CARs) or a fusion incorporating both activating and co-stimulatory properties (2nd generation CARs). In the

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case of 2nd-generation CARs, the design encompasses the CD3-ζ chain and the cytoplasmic domain of a co-stimulatory receptor, such as CD28, 4-1BB, CD80, or CD40L. The so-called 3rd-generation CARs integrate two co-stimulatory domains with an activation domain in their cytoplasmic region.

CAR-modified T cells are tumor-targeted T cells, enhanced T cell expansion and persistence within tumor microenvironment, thus rapidly evolve a potential cancer immunotherapy. The encoded expression of cytokines in CAR-T cells can further alter the tumor microenvironment, showing great enhancement for this approach. For example, CD19-targeted, CAR-modified T cells expressing IL-12 showed greater efficacy than CAR-modified T cells alone.

3) **About 4-1BB (CD137)**,
4-1BB (CD137) is a surface glycoprotein found on activated T lymphocytes, CD4+ and CD8+ T cells. It binds to its ligand on antigen-presenting cells, promoting T-cell antitumor activity. 4-1BB is widely used as a co-stimulatory domain in CAR design.

4) **About CD28**,
CD28, a transmembrane protein on T cells, provides co-stimulatory signals for T cell activation and proliferation. CD28 costimulatory domains in CARs enhance anti-malignancy efficacy, making it a common choice in CAR design.

5) **About CD3ζ**,
CD3ζ (T-cell receptor zeta) is expressed by T cells and NK cells. It together with T-cell receptor and CD3γ, δ, ε chain, forms the TCR-CD3 complex. It is a commonly used activation component of CARs transmitting activation signals to T cells after antigen binding.

6) **About CD19 and CD20**:
CD19 is a marker of in most B cell leukemias and lymphomas but not in any normal tissue other than the B cell lineage. CD20 is another B cell surface protein involved in B cell activation and differentiation. Both CD19 and CD20 is used to diagnose cancers that arise from this type of cell, and used as the target for CD19/CD20-targeted CAR-T therapies.

7) **About BCMA** (B-Cell Maturation Antigen):
BCMA is expressed on the surface of plasma cells, particularly mature B cells, and plays a role in B cell development. BCMA is a target in CAR-T therapy for multiple myeloma.

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8) **About HER2** (Human Epidermal Growth Factor Receptor 2):
Her2 is a receptor protein involved in cell growth and division. HER2, overexpressed in some breast cancers, is a target for CAR-T therapy in certain breast cancers.

9) **About CD22** (Cluster of Differentiation 22):
CD22 is a cell surface glycoprotein expressed on B cells. It is involved in B cell activation and regulation. CD22 is a target in CAR-T therapy for B-cell malignancies, especially in cases where CD19-targeted CAR-T cells may face challenges. CD22 CAR-T cells offer an alternative approach in certain B-cell lymphomas and leukemias.

10) **About HLA-A2** (Human Leukocyte Antigen A2):
HLA-A2 is a major histocompatibility complex (MHC) class I molecule involved in presenting antigens to cytotoxic T cells. HLA-A2 is not a traditional target for CAR-T therapy, as CARs typically recognize cell surface antigens rather than MHC molecules. However, CARs anti HLA is a promising strategy to induce immune tolerance in transplants.

11) **About TGFβ**:
TGF-β is a member of a family of proteins involved in various cellular processes, including cell proliferation, differentiation, migration, and apoptosis. It plays a key role in immune regulation by influencing the activity of immune cells and modulating inflammatory responses. Targeting TGF-β signaling pathways is being explored as a potential therapeutic strategy
TGF-β is often associated with immunosuppressive effects in the tumor microenvironment. High levels of TGF-β can contribute to immune evasion by inhibiting the activity of cytotoxic T cells and promoting the generation of regulatory T cells (Tregs). To counteract the immunosuppressive effects of TGF-β in the tumor microenvironment, the CAR-T cells are developed to resist TGF-β-mediated suppression.

2. Gentarget's CAR-T Lentivirus:

To generate genetically modified T cells targeting certain cancers, Gentarget Inc constructs CAR lentivectors expressing 2nd or 3rd generation chimeric antigen receptors (CAR) or a T cell receptor (TCR).

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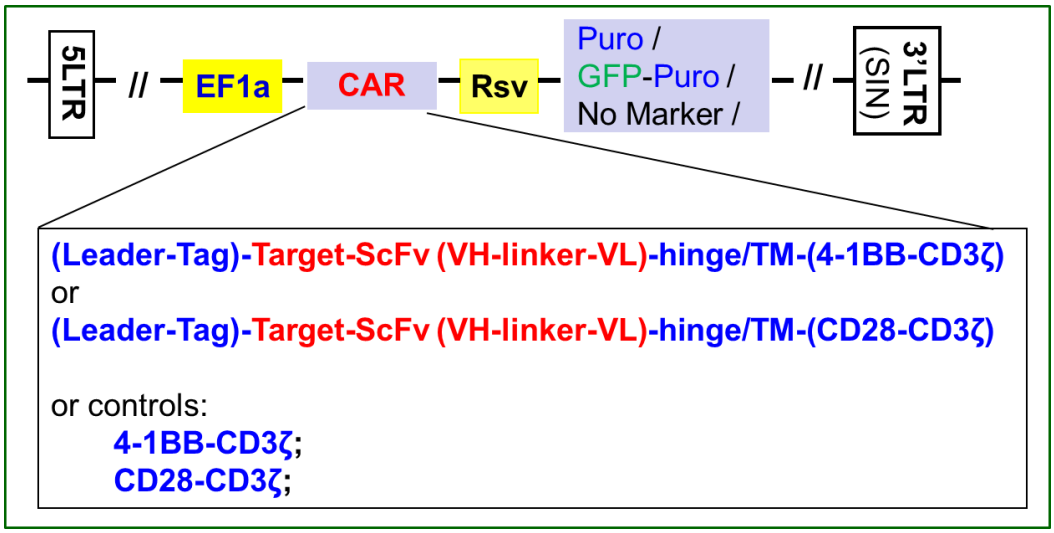
(1) **CAR-T Lentivector structure**:

Lentiviruses express the CAR construct under the enhanced EF1a promoter. The lentivector contains an antibiotic selection (Puromycin) or dual selection of GFP-Puromycin. The core structure is "(CD-Leader-(3xFLAG tag)-(anti-Target-ScFV: VH-Linker-VL)-(CD8-hinge_Trans-Membrane)-(4-1BB-CD3ζ or CD28-CD3ζ)".

See the core structure of the lentivirus in the following scheme.



Each "Anti-target antibody sequences" are derived from the verified clones according to published literatures, and expressed with N-terminal 3xFLAG tag (for detection of CAR expression, if desired, using anti FLAG reagents).

The stimulatory domain of either human 4-1BB (CD137) or human CD28 is constructed at downstream of Anti-target-antibody, and followed by the activation signal of CD3ζ.

Each CAR lentivector contains an antibiotic selection (Puromycin), or dual selection of GFP-Puromycin (so you can sort the positive transduced cells via GFP fluorescent signal or Puromycin killing), or No-selection as desired.

(2) **Validation of CAR-T specificity**:

CAR-T control lentivectors are constructed without a target-specific recognition domain for validation, still contains the corresponding selection marker (Puro, GFP-Puro, or no-selection).

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(3) **Lentivirus details**:

VSV-G pseudotyped, replication-incompetent CAR-T Lentivirus is provided in PBS solution at a titer of 1x10^8 IFU/ml, 200ul/vial. For more details, please see *FAQs for pre-made lentiviral particles* (.pdf).

3. Key features of Gentarget's CAR-T Lentivirus:

(1) Great tool to generate T cell CAR killing in cancer cells (Validation assay).
(2) High-titer lentivirus for high transduction rates in T cells.
(3) Strong promoter strength in T cells using the enhanced EF1a promoter.
(4) Detection of target expression by anti-FLAG antibody.
(5) Easy transduction verification by GFP fluorescent signal.
(6) Transduced cells can be sorted via a fluorescent signal or selected for antibiotic resistance.
(7) Ready-to-use: Add 50 to 100 µl 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 ul to 100 ul 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. CAR assay workflow (for reference only):

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(1) First, culture your desired T cells:

The established T cell lines or the isolated T cells or NK cells form patient's blood samples. 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. Use positive enrichment and negative removal.

(2) CAR or TCR cell stimulation / T cell activation:

1) Select the CARs or TCRs Lentivirus products that has your desired target. (For example, anti-CD19);
2) Apply the CAR or TCR lentivirus (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:

1) Target killing cell assay:
   Use a cell line the express the targeted antigen (FACS machine assay). For example: co-culture the CAR-T cells or TCR-T Cells with the target cell line, at 1:1 ratio, incubate in 37°C for one day; Measure the target cells killed (%) via FACS, or via fluorescent signal decrease if the target cells were labelled with a fluorescent marker.
2) Or, measure the secreted cytokines from cell supernatant: like TNF-a;

Notes: Because of the assay's research nature, and your cells' properties, 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 have advanced safety features (third-generation vectors with self-inactivation SIN-3UTR) and are replication incompetent. Use caution and handle lentiviral particles in a Bio-safety II cabinet. Always wear gloves. Refer to CDC and NIH guidelines for safety issues.

7. References:

1. T cells expressing CD19/CD20 bi-specific chimeric antigenreceptors prevent antigen escape by malignant B cells; Cancer Immunol Res. 2016 June ; 4(6): 498–508
2. A single chain Fv fusion molecule specifically recognizes the extracellular domain of the c-erbB-2 receptor. J Steroid Biochem Mol Biol. 1992 Sep;43(1-3):1-7;
3. Precision Engineering of an Anti-HLA-A2 Chimeric Antigen Receptor in Regulatory T Cells for Transplant Immune Tolerance. Front Immunol. 2021 Sep 20:12:686439

4. Identification and characterization of fully human anti-CD22 monoclonal antibodies. 1:3, 297-303; May/June 2009]; ©2009 Landes Bioscience.
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	Repoter 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.
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 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied 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

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Infectious Antigens	Llentivirus 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 verifeid 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-miNA 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.
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 fusioned 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

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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 ColorSwtich	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 inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic 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 lentivurs 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.