GenTarget Inc

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Premade Anti-CD19 CAR-T Lentivirus

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

Cat#	Product Name	Amounts
LVP1440	Anti-CD19-ScFv-4-1BB-CD3ζ (Puro)	200ul x ($1x10^8$ IFU/ml) in PBS solution, pre-mixed with polybrene.
LVP1441	Anti-CD19-ScFv-4-1BB-CD3ζ (Bsd)	
LVP1442	Anti-CD19-ScFv-4-1BB-CD3ζ (GFP-Puro)	
LVP1443	Anti-CD19-ScFv-4-1BB-CD3ζ (RFP-Puro)	
LVP1444	Anti-CD19-ScFv-4-1BB-CD3ζ (no marker)	
LVP1445	Anti-CD19-ScFv- CD28-CD3ζ (Puro)	
LVP1446	Anti-CD19-ScFv- CD28-CD3ζ (Bsd)	
LVP1447	Anti-CD19-ScFv- CD28-CD3ζ (GFP-Puro)	
LVP1448	Anti-CD19-ScFv- CD28-CD3ζ (RFP-Puro)	
LVP1449	Anti-CD19-ScFv-CD28-CD3ζ (no marker)	
CAR-ctr1	CAR negative control: 4-1BB-CD3ζ	
CAR-ctr2	CAR negative control: Anti-CD19-ScFv-4-1BB	
CAR-ctr3	CAR negative control: CD28-CD3ζ	
CAR-ctr4	CAR negative control: Anti-CD19-ScFv- CD28	

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

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1. Product Description:

1) About Lentivirus,
GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene delivery. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

2) What is CAR-T,
CARs (Chimeric Antigen Receptors) is the method to modify T cells to produce a special structure on their surface. The structure consists of two parties, the antigen-binding and the T cell activation. The Antigen-Binding, like an antibody, targets a tumor surface antigen. The antigen-binding moieties usually the scFv's derived from antibodies (for example, targeting a tumor surface antigen), or Fab segment, or nature ligands binding their cognate receptor. The activation part consists of a chimeric molecule activation domain (1st generation CARs), or a fusion containing both activating and co-stimulatory properties (2^{nd} generation CARs). The 2nd-generation CARs encompasses the CD3-ζ chain and the cytoplasmic domain of a co-stimulatory receptor such as CD28, 4-1BB, CD80 CD40L. The so-called 3^{rd}- generation of CARs embed two co-stimulatory domains combined with an activation domain in their cytoplasmic domain.

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 CD19,
CD19 is a marker of in most B cell leukemias and lymphomas but not in any normal tissue other than the B cell lineage, CD19 is used to diagnose cancers that arise from this type of cell, and used as the target for CD19-targeted therapies.

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4) About 4-1BB (CD137),
4-1BB (CD137) is a surface glycoprotein present on activated T Lymphocytes, CD4+ and CD8+ T cells. It binds to its ligand expressed on antigen-presenting cells (macrophages and activated B cells). It promotes T-cell antitumor activity. 4-1BB is one of the most widely studied co-stimulatory domain used in CAR design.

5) About CD28,
CD28 is transmembrane protein expressed on T cells. It provides co-stimulatory signals for inducing T cell activation and proliferation. CD28 costimulatory domains in CARs led to enhanced anti-malignancy efficacy. CD28 is widely used as the costimulatory domain in CARs.

6) 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. CD3-zeta is the most commonly used activation component of CARs. It transmits an activation signal to the T cell after the antigen is bound. It can be coupled with additional co-stimulatory signaling for the complete activation.

2. Gentarget's CAR-T Lentivirus:

To generate the genetically modified T cells that kill certain types of cancer cells, Gentarget Inc constructs the tumor-specific CAR lentivectors, expressing the 2nd or 3rd generation of chimeric antigen receptor (CAR) or a T cell receptor (TCR).

CAR-T targeted on CD19: Those lentivirus express the CAR construct of "anti-CD19-ScFV (VL-VH)-CD8 hinge-4-1BB-CD3ζ", or "anti-CD19 ScFV (VL-VH)--CD8 hinge-CD28-CD3ζ", under the enhanced EF1a promoter that tested strong in T cells. A signal leader with 3x FLAG tag, is constructed in front of Anti-CD19 ScFV. The Anti-CD19 is the verified clone FMC63. The stimulatory domain of either human 4-1BB (CD137) or human CD28 is constructed at downstream of Anti-CD19, and followed by the activation signal of CD3ζ.

For the validation of CAR-T specificity, two types of CAR-T controls are constructed by either remove Anti-CD19 ScFV recognition domain or remove CD3ζ activation domain.

To avoid any unwanted immnuo-antigen, the lentivirus does not contain any antibiotic marker, or as desired, contains an antibiotic selection marker, or

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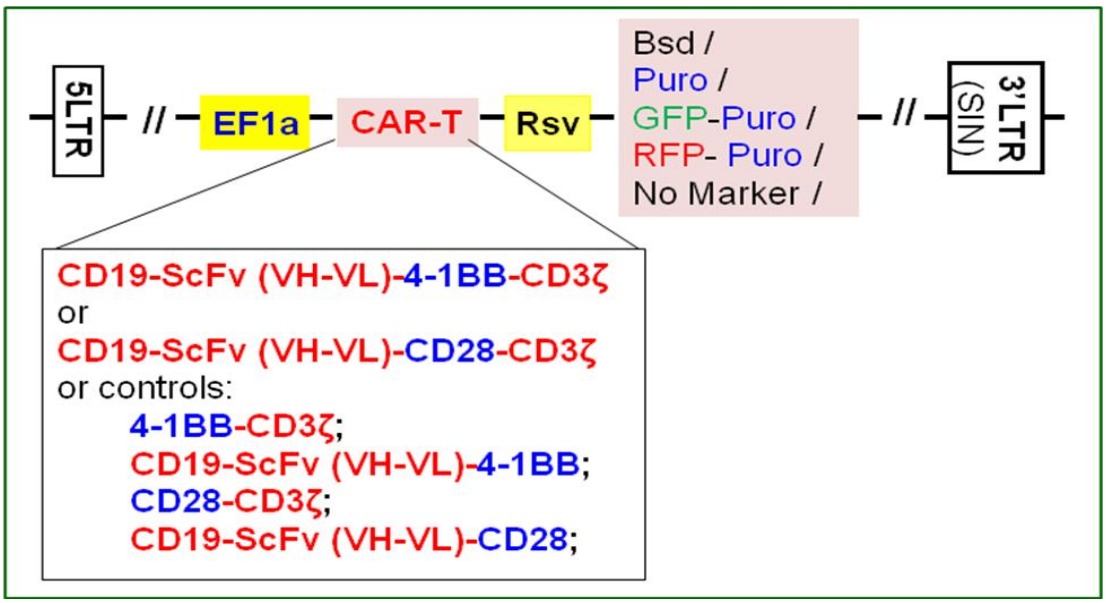
Fluorescent-Antibiotic fusion dual selection marker under Rsv Promoter. See the core structure of the lentivirus in the following scheme.



The VSV-G pseudotyped CAR-T Lentivirus are generated from 293T cells, and the concentrated lentivirus is provided in PBS solution at the titer of 1x108 IFU/ml, as 200ul aliquots.

For more details, please see *FAQs for pre-made lentiviral particles* (.pdf).

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

- Idea as the validation assay for T cell CAR killing in CD19 positive cancer cells;
- High titer lentivirus for the high transduction rate in T cells;
- High promoter strength in T cells by enhanced EF1a promoter;
- Easy transduction verification by the GFP or RFP signal (when desired);
- Dual selection markers: transduced cells can be sorted via a fluorescent signal or selected for antibiotic resistance (when desired);
- Ready to use: simply add 50 µl into your cell culture in a 24-well plate, and leave the virus on for 48hr to 72 hours. (Note: depending upon your specific needs, you may transduce with different MOIs for different levels of expression.)

4. Transduction Protocol in T Cells (lymphocyte):

Note: Pre-made lentivirus is provided ready to use, simply added into your cell culture. The amount of virus to add depends on cell type. For quick transduction, add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75% or at the cell number as 1 million/ml for suspension cells. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy when applicable. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting.

1) Seed 0.5 ml of T lymphocyte cells (CD4+ and CD8+) in complete medium, into one wall in 24 well/plate, at the cell concentration of 1 million cells/ml, at the time of lentivirus transduction (i.e. 0.5 million cells/well);
2) Thaw the Lentivirus at room temperature, and add 50 ul of the CAR-T lentivirus into each well in 24 w/p. You can scale up the transduction when needed for larger culture volume; No need to add polybrene as the provided lentivirus are pre-mixed with 10x polybrene.
3) Return cells to 37°C, CO_2 incubator;

   Note: Try to avoid freezing and thawing. If you do not use all of the lentivirus at one time, you may re-freeze the virus at -80 ^{o}C for future use; lentivirus titer will decrease by ~10% for each freeze/thaw cycle.

4) At 48hr to 72hr post transduction, transfer the cells to flask in completed medium (when applicable, with puromycin selection) for the transduced cell enrichment and expansion; or analysis via FAC.
5) When cell grow to the desired cell number (about one more week culture), check the transduction rate by fluorescence microscopy or by flow cytometry, or simply go for the validation of the anti-CD19 CAR-T assays (For example, use our *CD19 expression cell lines* as targeted killing assay which can be measured via GFP signal decay via plate readout format or via FAC.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;

5. Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Bio-safety II cabinet. Ware glove all the time at

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handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

6. References:

1. David M; The journal of immunology,2015
2. Porter DL et al; N Engl J Med. Aug 25 2011;
3. Kochenderfer JN, et al; J Clin Oncol. Feb 20 2015.
4. NIH Guidelines for Bio-safety Considerations for Research with Lentiviral Vectors. (Link).
5. CDC guidelines for Lab Bio-safety levels (Link).

7. Warranty:

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

8. Related products: GenTarget's pre-made lentivirus product category.

Lentivirus Category (click to see)	**Product Description**
Target Expression	Premade lentivirus express a **human, mouse or rat** gene with Fluorescent-Antibiotic fusion dual selection.
Luciferase expression	Premade lentivirus express all kinds of luciferase**: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc,** with different fluorescent and antibiotic selection.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP/niRFP /unstable GFP, etc.**
Cytoskeleton Imaging	Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing by **CRISPR**
Fluorescent Fusion target	Lentivirus express the "**Fluorescent-Target**" fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan Fluorescent Protein, demonstrating the target's functionality and localization

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CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
SEAP Reporter	**SEAP** (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
TetR repressor expression	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	Lentivirus express the reverse tetraccycline transcription activator gene, rtTA-M2 with different selection.
Pathway Reporter	Different Report lentivirus (**Luc, RFP, GFP, SEAP**) under a pathway specific response promoter.
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.
Infectious Antigens	Lentivirus express all kinds of infectious antigens.
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
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
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
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

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	target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.

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