



## Pre-made Reporter Lentivirus for CD44CR1 Signal Pathway

Cat#	Product Name	Amounts
<a href="#">LVP812-P</a>	CD44CR1- <b>GFP</b> (Puro) Lentiviral particles	200ul, (1 x 10 <sup>7</sup> IFU/mL) containing 10x Polybrene
<a href="#">LVP813-P</a>	CD44CR1- <b>RFP</b> (Puro) Lentiviral particles	
<a href="#">LVP814-P</a>	CD44CR1- <b>Luc</b> (Puro) Lentiviral particles	
<a href="#">LVP815-P</a>	CD44CR1- <b>Rluc</b> (Puro) Lentiviral particles	
<a href="#">LVP812-B</a>	CD44CR1- <b>GFP</b> (Bsd) Lentiviral particles	
<a href="#">LVP813-B</a>	CD44CR1- <b>RFP</b> (Bsd) Lentiviral particles	
<a href="#">LVP814-B</a>	CD44CR1- <b>Luc</b> (Bsd) Lentiviral particles	
<a href="#">LVP815-B</a>	CD44CR1- <b>Rluc</b> (Bsd) Lentiviral particles	
<a href="#">LVP812-N</a>	CD44CR1- <b>GFP</b> (Neo) Lentiviral particles	
<a href="#">LVP813-N</a>	CD44CR1- <b>RFP</b> (Neo) Lentiviral particles	
<a href="#">LVP814-N</a>	CD44CR1- <b>Luc</b> (Neo) Lentiviral particles	
<a href="#">LVP815-N</a>	CD44CR1- <b>Rluc</b> (Neo) Lentiviral particles	
<a href="#">LVP812-R</a>	CD44CR1- <b>GFP</b> ( <b>RFP</b> ) Lentiviral particles	
<a href="#">LVP814-R</a>	CD44CR1- <b>Luc</b> ( <b>RFP</b> ) Lentiviral particles	
<a href="#">LVP815-R</a>	CD44CR1- <b>Rluc</b> ( <b>RFP</b> ) Lentiviral particles	
<a href="#">LVP813-G</a>	CD44CR1- <b>RFP</b> ( <b>GFP</b> ) Lentiviral particles	
<a href="#">LVP814-G</a>	CD44CR1- <b>Luc</b> ( <b>GFP</b> ) Lentiviral particles	
<a href="#">LVP815-G</a>	CD44CR1- <b>Rluc</b> ( <b>GFP</b> ) Lentiviral particles	

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

### Product Description:

**Lentiviral system** is a gene delivery tool using lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and



knockdown. 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.

## **CD44R1 signaling pathways:**

The CD44 antigen is a multi-structural and multifunctional cell surface glycoprotein involved in cell proliferation, cell differentiation, cell migration, angiogenesis. CD44 is highly expressed in many cancers and has been implicated in tumorigenesis and metastasis. The up-regulation of CD44 has served as a marker for tumor initiating cells in breast cancer and other cancer types.

The mechanism that leads to a high level of CD44 expression is that some *trans*-acting factors, like NFkB or AP-1, interact with a *cis*-element, the conserved region 1 (CR1), located upstream of the CD44 promoter. Inhibition of NFkB can lead to down-regulation of CD44 expression. This CD44 expression regulation pathway provides the potential therapeutic targets.

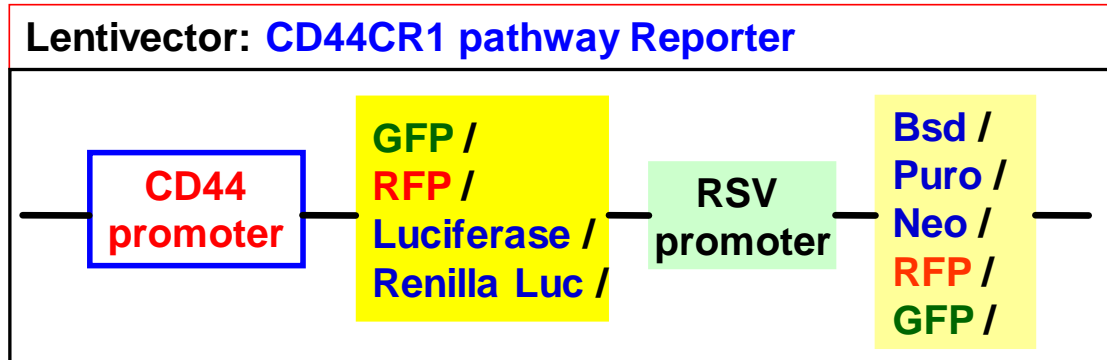
## **Premade CD44CR1 Pathway Signaling Lentivirus:**

GenTarget developed a set of reporting lentivirus to monitor CD44 promoter pathway regulation. Those lentivirus expresses a report (**firefly Luciferases, Renilla luciferase, GFP or RFP**) under CD44 promoter containing its CR1 region ([CD44CR1](#)). The report's expression will be increased when a trans-activator, like NFkB, binds to this promoter region. The report signal level is the result of activation the CD44 promoter. The reporter's signal can be easily and rapidly readout via all types of assays (luciferase assay or by fluorescent microscope or FACS).

The premade, ready-to-use reporter lentivirus provides an easier, sensitive and quantitative tool to monitor the activity of CD44CR1 signaling pathways in virtually any mammalian cell type. It also allows to generate your own reporting cell line in your desired cell type for study or screen of pathway specific gene-knockdown, over-expression, or chemical / drug/protein treatment in the cell based assay.



see lentivector's core structure scheme below.



Lentivirus are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

The lentivirus are ready and easy to use, simply add 50ul into one well of your cell culture in 24-well plate, and select or sort the positive transduced cells at 2-3 days post virus transduction (for sensor cell line assay). Or simply go for Androgen signal induction without the selection (for transient assay). The readout can be easily monitored by luciferase assay or via the Fluorescent microscope or readers depending on product report type.

### Key Application for Pathway Signaling Lentivirus:

1. Create signal pathway specific cell lines which can provide a High-throughput, live cell based assays for signal transduction tests;
2. Identify or validate the signaling pathway specific drugs (drug discovery and validation);
3. Analyze the pathway-specific responses to proteins, peptides, or hormones;
4. Analyze the pathway-specific responses to gene activation, over-expression, knockdown, knockout, or mutagenesis;
5. Screen for pathway-specific stimulus or for the transcriptional activators that response to specific pathway's TRE elements;
6. Direct report's cell specific expression where the CD44CR1 pathway can be activated.

For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

### Transduction Protocols:



**Note:** Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50  $\mu$ l of virus into each well of 24-well-plate where cell density is 50% to 75% (It equivalent to a MOI=50 for most cell types at such conditions). After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic for selection, or perform fluorescence cell sorting.

### Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

**Note:** at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at  $0.5 \times 10^5$ /ml x 0.5ml in a well of a 24-well plate.

### Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.
- Return cells to 37°C, CO<sub>2</sub> incubator.

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

### Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava). You can now treat the cell for signal pathway assay.

### Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines). The selected stable cells will be used for signal pathway assay with desired treatment.

**Next:** Treat the cell with signal pathway inducer, and analyze the pathway reporter expression (Fluorescent readout or luciferase assay).



## Safety Precaution:

GenTarget lentiviral particles adapt 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. Wear glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

## References:

1. Bioessays. 2007 Dec;29(12):1227-38.;
2. J Hematol Oncol. 2018; 11: 64;
3. PLoS One. 2012; 7(11): e50867.

## 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.

### Note: Filter wavelength settings:

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

**Related Products:** GenTarget's pre-made lentivirus product category.

<b>Product Category</b>	<b>Product Description (please click category name to see product's pages)</b>
<a href="#">Human, mouse or rat ORFs</a>	Premade lentivirus expressing a <b>human, mouse or rat</b> gene with RFP-Blastididin fusion dual markers.
<a href="#">Fluorescent markers</a>	Premade lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP</b> .
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla</b> with different antibiotic selection markers.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
<a href="#">CRISPR /hu CAS9</a>	Premade lentivirus express humanized wild-type <b>Cas9</b> endonuclease for genomic editing with <b>CRISPR</b>
<a href="#">TetR inducible</a>	Premade lentivirus expressing <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.



<a href="#">expression repressor</a>	
<a href="#">iPS factors</a>	Premde lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLF4</b> ) factors with different fluorescent and antibiotoxic markers
<a href="#">T-antigen Expression</a>	Express <b>SV40 large T antigen</b> with different selection markers
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent marker <b>GFP/RFP/CFP was sub-cellular localized</b> in different cell organelle for living cell imaging.
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>- galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.