Ready-to-use Lentiviral Particles for monitoring CRE recombination reaction

Catalog Number	Product Name / Description	Amount*
LVP460-Puro	**LoxP GFP/RFP ColorSwitch lentivirus (Puro):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **puromycin** antibiotic selection marker under Rsv promoter.	200ul /per vial x ($1x10^7$ IFU/ml) in DMEM medium with 10% FBS and 10x polybrene
LVP460-Neo	**LoxP GFP/RFP ColorSwitch lentivirus (Neo):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **Neomycin** antibiotic selection marker under Rsv promoter.	
LVP460-Bsd	**LoxP GFP/RFP ColorSwitch lentivirus (Bsd):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **Blasticidin** antibiotic selection marker under Rsv promoter.	
LVP460-Puro-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (Puro):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **puromycin** antibiotic selection marker under Rsv promoter.	200ul /per vial x ($5x10^7$ IFU/ml) in PBS
LVP460-Neo-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (Neo):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **Neomycin** antibiotic selection marker under Rsv promoter.	
LVP460-Bsd-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (Bsd):** Pre-made lentiviral particles expressing "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter. It also contains a **Blasticidin** antibiotic selection marker under Rsv promoter.	

*** Titers may vary lot-to-lot. Please refer to the titer stated on the pack size description in our website.**

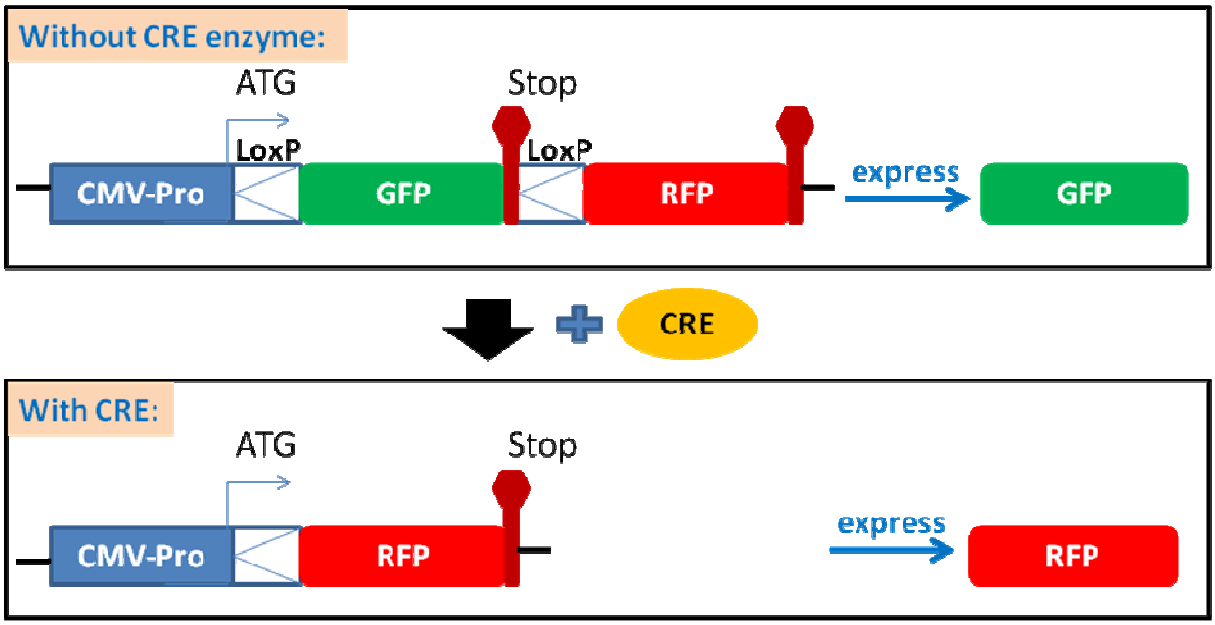
Storage: < -70 °C, avoid repeat freeze/thaw cycles. Stable for > 6 months.

Product Description

Lentiviral system is a gene delivery tool using lentivectors for gene expression or knockdown. Lentivectors are HIV-1 (Human Immunodeficiency Virus 1) derived plasmids, used to generate lentiviral particles (lentivirus) that can be transduced into virtually all kinds of mammalian cell types or organs, including stem cells, primary cells and non-dividing cells both ***in vivo*** and in **cell culture** system. Particles stably integrate into the transduced cells' genome for long term expression. Therefore, lentivirus holds unique promise as gene transfer agents.

CRE recombinase, from bacteriophage P1, catalyzes recombination between 34 base pair target sequences, named Lox sites. CRE-Lox recombination is a special type of site-specific recombination, and widely used to delete loxP-flanked chromosomal DNA sequences at high efficiency *in vivo*. By inserting a "LoxP-flanked expression target" into host's genome, we can control this target expression with the help of CRE recombinase. In other words, the target's expression occurs before CRE enzyme applied. And after CRE enzyme is applied, CRE deletes the LoxP flanked target segment and stops the target expression. In the mean time, as desirable, this genomic alternation can activate the 2nd target at downstream of the deleted segment. The CRE recombination provides an excellent tool for conditional gene targeting in transgenic animal modeling to link genotypes (alterations in genomic DNA) to the biological outcomes (phenotypes).

To monitor the effectiveness of CRE recombination event, AMSBIO provides **pre-made CRE reporting lentivirus** that test or monitor the CRE recombination efficacy *in vivo* or *in cell* culture. This lentivirus was engineered to constitutively express the "LoxP-GFP-stop-LoxP-RFP-Stop" cassette under a super CMV promoter. (see the expression cassette scheme below). It constantly monitor the occurrence of CRE enzyme in the environment, and report the presence of CRE enzyme or *via* a color switch mechanism, which provides an easy, fast and convenient indicator for the CRE recombination event.



AMSBIO provides the CRE reporting lentivirus with different antibiotic selection marker as **Puromycin, Neomycin or Blasticidin**. This selection marker was expressed under a RSV promoter (not showed in the scheme above). Therefore, you can easily select the positive transduced cells (expressing the CRE detection cassette) *via* a specific antibiotic marker, blasticidin (Bsd), Puromycin (puro) or Neomycin (Neo).

How it works:

The CRE reporting lentiviruses are used to monitor or confirm the efficiency of CRE recombination *in vivo*. It is a great method and easy tool to verify the performance of your CRE enzyme (your CRE expression plasmids, or pre-made CRE expression lentivirus, or purified CRE enzyme) *in vivo* conditions. It is also a control indicator to verify your CRE-loxP based system.

This lentivirus demonstrates strong GFP fluorescent signal after applied into any mammalian cells but without RFP fluorescent signal. The downstream RFP ORF was not expressed* because of the stop codon after the GFP. Once the CRE protein was present in nuclear, the CRE excises / deletes the DNA fragment between two loxP sites, which removes the stop codon after the GFP (see the scheme image above). As a result, the RFP ORF is then expressed under the CMV promoter, and the cells switches to RFP fluorescent**. The RFP signal can be easily monitored via fluorescent cell sorting (for the ratio between GFP and RFP cells), or visualized under microscope, or measured the fluorescent intensity by a meter or reader with GFP, RFP filter sets.

Note:
***:** Like any mammalian pol II promoters, the CMV promoter could seek any possible ORFs after the designed stop codon, and may express the far end ORF (the RFP in this case), which is considered the basal RFP signal or leaking RFP signal here.
****:** It is not possible to deliver the CRE into all cells, therefore, there are still GFP only positive cells even after the CRE applied. Also, because some cells may integrate multiple copies of LoxP-GFP-Loxp-RFP cassettes, and the CRE recombination is not possible at 100% rate for all sites at each cell, therefore, there are many cells demonstrate both GFP and RFP signal after addition of CRE enzyme. The main observation here is to see the dramatically increase of RFP positive cells which reflects the CRE recombination rate.

Application protocol (for reference only):

1. Adhesive cells Transduction Protocols:

Note: A quick transduction protocol is: add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after virus addition (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells *via* fluorescent signal. Then, select the cells by antibiotics.

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 50% ~75% confluent.)
For example, seed Hela cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate;

Day 1: Remove the culture medium. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO^2 incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80oC for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At ~72hr after transduction, check the transduction rate *via* fluorescence image with a suitable filter under fluorescent microscope, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine). Note: You should only see GFP signal at this stage before you apply CRE enzyme to the cells.

Day 3 + : Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.).

CRE enzyme delivery: The selected cell should demonstrate strong GFP signal and should have no RFP signal. After the cell selection, the cells are ready used as an indicator cell line for CRE recombination activity.

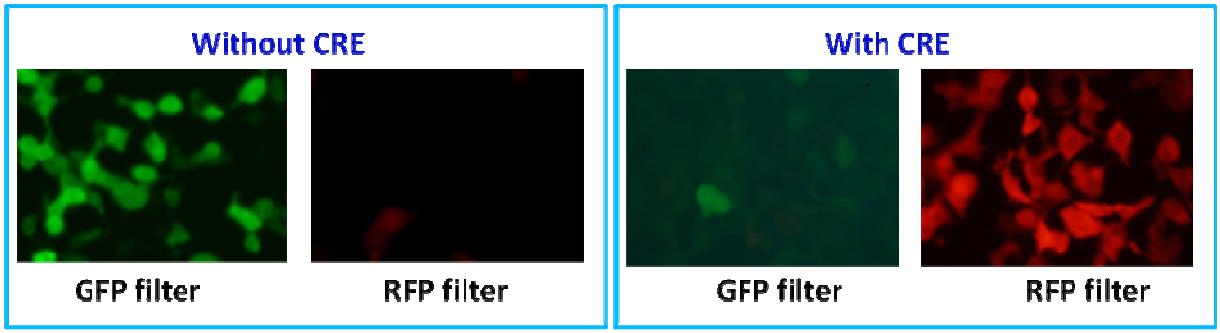
- **Apply the CRE enzyme into the cells** (which can be achieved by infected cell with CRE expression lentivirus, or by regular lipid-transfection of a CRE expression plasmid, or even simply by adding purified penetrating CRE protein enzyme. (**Note**: AMSBIO provides ready-to-use **CRE expression lentivirus** (http://www.amsbio.com/datasheets/Ready-to-use_Lentiviral_Particles_for_Luciferase_Expression.pdf) with different antibiotic selection marker for CRE delivery into cells).
- Put cells in normal culture conditions for **48-72 hours**.
- **Detect CRE recombination reaction**: The RFP signal will gradually show up and peak at 48 hours or longer times (dependent upon CRE delivery methods) after the CRE delivery. The RFP/GFP cell population ratio or the RFP signal intensity reflects the CRE-LoxP recombination efficiency (rate). You can sort the cell by FACS machine, other meters, or visualize the RFP positive cell under fluorescent signal.

2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO^2 incubator if necessary;
2. Measure cell density. When cell grow to ~3 x 10^6 cell/ml, measure cell viability (should be > 90%), then diluted cells into 1 x 10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **50 to 100ul virus per 0.5 ml of cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in CO^2 incubator.
5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
6. You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620. Fusion marker has slightly shifted wavelength, but no need for filter changes.).

Sample images of CRE-loxP recombination detection:



Left panel / without CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was created by LoxP460-Neo particles, cultured in 24-well-plate. Images were taken under microscope with GFP filter set (Ex 490nm/Em 525nm) and RFP filter set (Ex 545nm/Em 620nm).
Right panel / with CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was was created by LoxP460-Neo particles, cultured in completed in 24-well plate. 50ul of CRE expression lentiviral particle (Cat#: **LVP339)** was added into the cells in one well. Images were taken at ~ 72 hours after the addition of CRE expression lentivirus.

Related Products:

Product Category	Product Description
nuclear permeable CRE	Premade lentivirus for expressing nuclear permeable **CRE recombinase** with different fluorescent and different antibiotic selection markers
Luciferase expression	Premade lentivirus for **Firefly**-luciferase II, **Renilla**-luciferase, **Gaussia**-luciferase and **Cypridina**-luciferase with all different fluorescent and antibiotic markers
iPS factors	Premade lentivirus for human and mouse iPS (**Myc, NANOG, OCT4, SOX2, KLF4**) factors with different fluorescent and antibiotic markers
Human and mouse ORFs	Premade lentivirus for **hundred of human and mouse ORFs** with RFP-Blastididin fusion dual markers
Living cell imaging	Premade lentivirus particles for Cell Organelle imaging including **Nucleus, Cytoplasm, Endoplasmic Reticulum, Golgi, Mitochondria, Nuclear membrane, Peroxisome, Plasma membrane, Microtubule, Chromatin, Annexin, Actin, Connexin**, and more
shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more). Consult our custom service page to have your own shRNA lentivirus made: http://www.amsbio.com/custom-lentivirus-service-inducible-shRNA-lentivirus.aspx
Negative controls	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

Safety Precaution:

Those lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the ready-to-use lentiviral particles are 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 when handling Lentiviral particles!

Please refer CDC and NIH's guidelines for more details regarding to safety issues:
http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html

References:

1. J Virol. 2000 November; 74(22): 10778–10784.
2. Hum Gene Ther (2003) 14: 1089-105.
3. Mol Ther (2002) 6: 162-8.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors

Warranty:

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

For general information about our
ready-to-use or custom made lentiviral particles, please consult:
http://www.amsbio.com/Lentivirus.aspx

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85