Expression Lentivirus for Detection of CRE Recombination Reactions

Catalog Number	Product Name / Description	Amount
LVP460-Puro-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (suCMV, Puro):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter, containing **puromycin** antibiotic selection.	**200ul** /vial x [**1 x10^8** IFU/ml, in PBS solution, premixed with 10 x Polybrene /60 ug/ml]
LVP460-Neo-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (suCMV, Neo):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter, containing **Neomycin** antibiotic selection.	
LVP460-Bsd-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (suCMV, Bsd):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under suCMV promoter, containing **Blasticidin** antibiotic selection.	
LVP1332-Puro-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (EF1a, Puro):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under **EF1a** promoter, containing **puromycin** antibiotic selection.	
LVP1332-Neo-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (EF1a, Neo):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under **EF1a** promoter, containing **Neomycin** antibiotic selection.	
LVP1332-Bsd-PBS	**LoxP GFP/RFP ColorSwitch lentivirus (EF1a, Bsd):** Lentivirus express "**LoxP-GFP-Stop-LoxP-RFP-Stop**" cassette under **EF1a** promoter, containing **Blasticidin** antibiotic selection.	

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

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Product Description:

1. Introduction:

The 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.

CRE recombinase, from bacteriophage P1, catalyzes recombination between 34 base-pair target sequences called lox sites and can join individual plasmids containing lox sites. CRE recombination provides an excellent tool for conditional gene targeting in transgenic animal models by linking genotypic alterations to the biological outcomes (phenotypes).

By inserting a "LoxP-flanked expression target" into a host's genome, target expression can be controlled via CRE recombinase. Expression of LoxP-flanked target genes may occur prior to the addition of CRE enzyme; when CRE is applied, it deletes the LoxP flanked target segment and stops the target expression. Simultaneously, CRE-mediated recombination can activate expression of a second target downstream from the deleted segment.

AMSBIO provides **CRE reporting lentivirus** for easy, fast and convenient testing and monitoring / detecting of CRE recombination efficiency *in vivo* and *in vitro*. This lentivirus has been engineered to constitutively express the "**LoxP-GFP-stop-LoxP-RFP-Stop**" cassette under either an enhanced CMV promoter or an enhanced EF1a promoter. CMV promoter provides the highest expression level in most cell types, EF1a promoter tends not to get silenced in long-term culture. (See the expression cassette scheme below).

Those products detect the occurrence of CRE-mediated recombination events via a "color switch" mechanism, thereby providing an essay, fast and continual monitoring for the presence of CRE or CRE recombination event.

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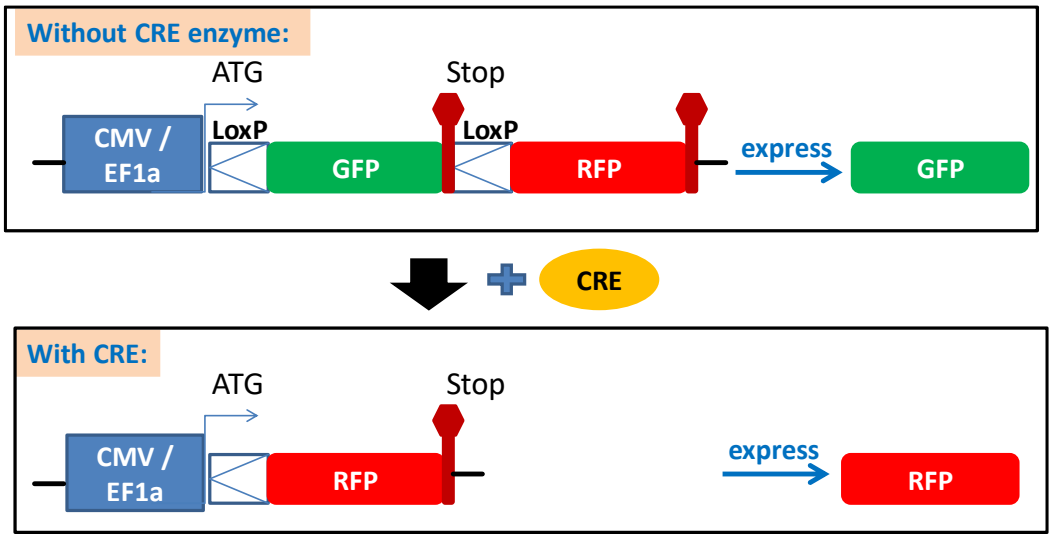


2. Desired Promoter and Selection Marker:

(1) The color-switch cassette is driven by either **CMV** promoter or **EF1a** promoter. You can pick the best promoter suitable for your cell types. The CMV give the highest expression level in most cell types. The EF1a promoter has medium to high express level and not subject to promoter silence.

(2) Those CRE reporting lentivirus contains an antibiotic selection of **puromycin, neomycin, or blasticidin**. The selection is expressed under an RSV promoter (not shown in the schematic above). These markers allow easy selection for transduction positive cells by antibiotic killing.

3. Product Formats:

The LoxP ColorSwtich lentivirus are provided as at **200 µl/vial** in two formats:

(1) DMEM medium with 10 % FBS and 60 µg/ml polybrene (10x)

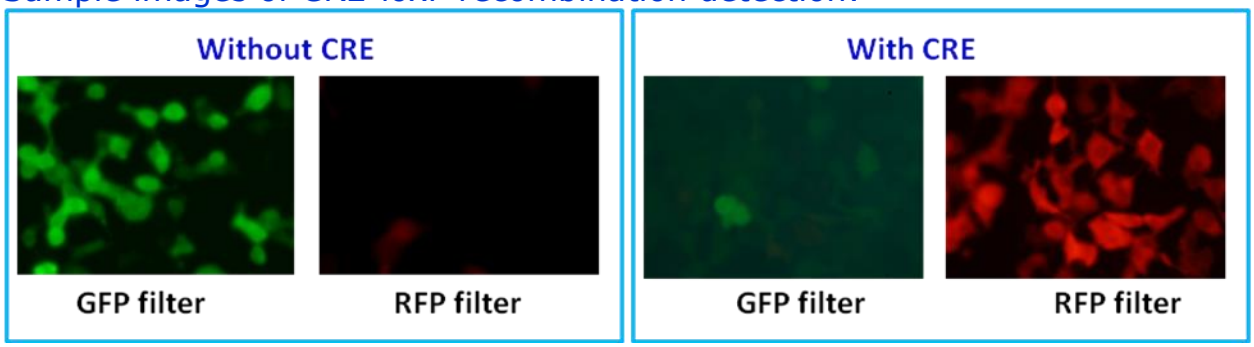
(2) Concentrated lentivirus provided in PBS solution, which is best for *in vivo* applications, cell cultures requiring serum-free conditions, or hard-to-infect cells.

4. How ColorSwtich Reporting Works:

The CRE reporting lentivirus is used to monitor or detect the efficiency of CRE recombination *in vivo*. It is an easy and effective tool for verifying the performance of CRE-loxP systems *in vivo*.

The CRE reporting lentivirus demonstrates the strong GFP fluorescence after infection into mammalian cells, but does not show an RFP fluorescence signal. Once the CRE protein is present in the nucleus (which can be delived by CRE expression lentivirus, or lipid transfected CRE expression plasmid), CRE enzyme excises/deletes the DNA fragment between two loxP sites. As a result, the GFP is removed and the downstream RFP is expressed. You will observe a color switch from GFP to RFP fluorescence. The ratio of RFP / GFP cells can be easily monitored via fluorescence cell sorting, visualized by microscopy, or the fluorescence intensity measurement by fluorometer. See the sample results below.

Sample images of CRE-loxP recombination detection:



(Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).

Left panel / without CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was created by LoxP460-Neo particles, cultured in a 24-well-plate. Images were taken with a GFP filter set (Ex 490nm/Em 525nm) and an RFP filter set (Ex 545nm/Em 620nm).

Right panel / with CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was created by LoxP460-Neo particles, cultured in completed in 24-well plate. 50 µl 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.

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Notes:

*Like any mammalian pol II promoter, the CMV promoter seek any possible ORFs, and in some cell types, it can slightly express the 2nd ORF (the RFP in this case) which is considered the basal or leaking RFP signal.

** If CRE does not deliver into all cells, you may see some GFP positive cells after the CRE addition.

***Also, because some cells may integrate multiple copies of the LoxP-GFP-LoxP-RFP cassette, you may observe both GFP and RFP signals in a few cells after the addition of CRE recombinase. The important observation is the dramatic increase in RFP positive cells following addition of CRE. And the RFP/GFP intensity ratio reflects the CRE recombination rate.

Application protocol:

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 added (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. Or simply 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.).

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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** 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 showed up and peaked 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.

Safety Precaution:

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

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References:

1. Sauer, B. (1987) "Functional expression of the Cre-Lox site-specific recombination system in the yeast *Saccharomyces cerevisiae*", *Mol Cell Biol* **7**: 2087-2096
2. Stanislaw J. Kaczmarczyk and Jeffrey E. Green. Nucleic Acids Res. 2001 June 15; 29(12): e56.

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.

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