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Data Sheet

Firefly Luciferase Lentivirus (UbC Promoter)

Catalog #: 79880

Product Description

The Firefly Luciferase Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by a UbC (ubiquitin C) promoter (Figure 1). The ubiquitin C promoter is useful for high-level expression across a broad range of species and tissue types.

Applications

1. Ideal as a positive control for transduction; useful for transduction optimization.
2. Generation of stable cell line expressing firefly luciferase with puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 μ l x 2) of firefly luciferase lentivirus at a titer $\geq 5 \times 10^6$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

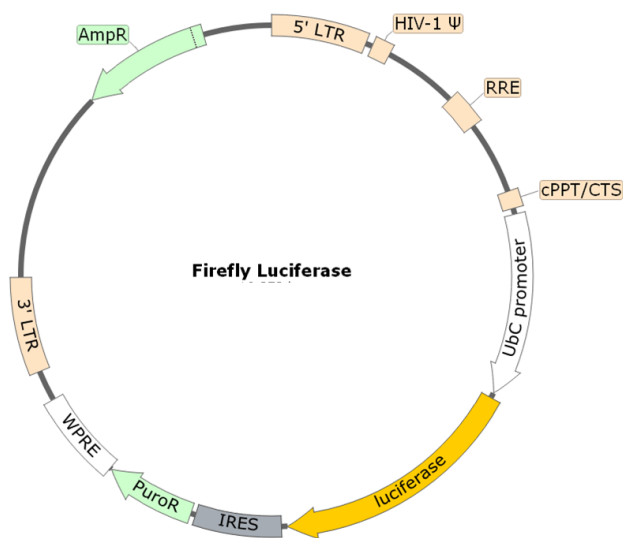


Figure 1. Schematic of the lenti-vector used to generate the firefly luciferase lentivirus

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Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Assay Protocol

The following protocol is a general guideline for transducing JIMT-1 cells using firefly luciferase reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

1. Day 1: Harvest JIMT-1 cells from culture and seed cells at a density of 5,000-10,000 cells per well into a white opaque 96-well microplate in 50 µl of JIMT-1 growth medium. Incubate cells at 37°C with 5% CO₂ overnight.
2. Day 2: To each well add 10 µl of firefly luciferase reporter lentivirus. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO₂ for 18-24 hours.

Alternatively, seeding cells and the transduction can be performed at on the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh JIMT-1 growth medium to each well.

If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing the medium.

4. Incubate at 37°C with 5% CO₂ for 16-24 hours.
5. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

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Important Notes:

1. To generate the firefly luciferase reporter stable cell line, on day 4 remove JIMT-1 growth medium and replaced it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.
2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:
 - 1) Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.
 - 2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

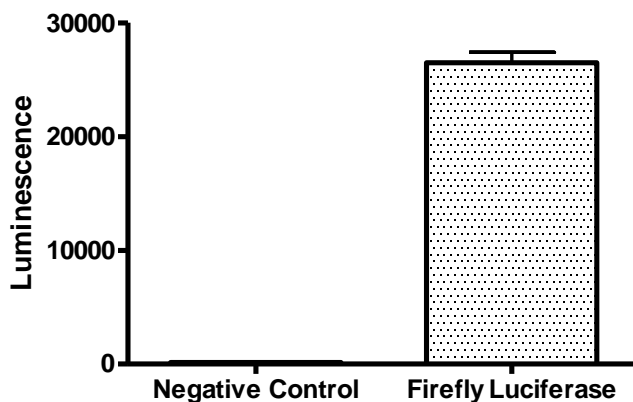


Figure 2. Luciferase activity in JIMT-1 cells transduced with firefly luciferase lentivirus (UbC promoter). Approximately 10,000 JIMT-1 cells/well were transduced with 100,000 TU/well firefly luciferase lentivirus or negative control lentivirus (BPS Bioscience #79578). After 66 hours of transduction, medium was changed to JIMT-1 growth medium. The luciferase assay was performed using the ONE-Step™ Luciferase assay system (BPS Bioscience, #60690), following the recommended protocol in the user manual. The results are shown as the raw luminescence reading.

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Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml

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