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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Renilla Luciferase-eGFP Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. The particles contain Renilla Luciferase and eGFP (green fluorescent protein) (RLuc-P2A-eGFP) driven by an EF1A promoter. The lentiviruses also transduce a hygromycin selection marker (Figure 1). The Renilla luciferase and eGFP proteins are under the EF1A promoter and are co-expressed in transduced cells, allowing greater flexibility of detection of the transduced cells.

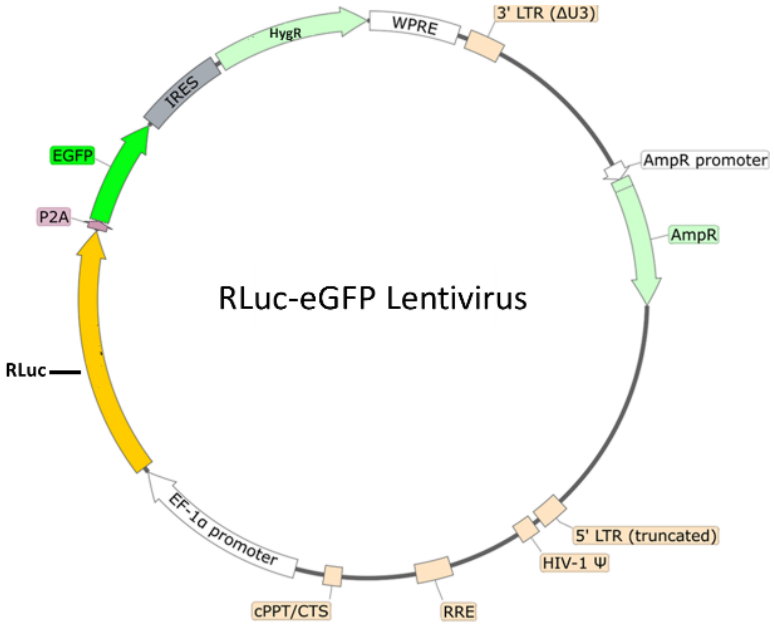

Figure 1. Schematic of the lenti-vector used to generate the Renilla Luciferase- eGFP Lentivirus.

Background

Renilla Luciferase, also known as RLuc, or Renilla-luciferin 2-monooxygenase, is a bioluminescent protein identified in *Renilla reniformis*. The chemical reaction that leads to emission of luminescent involves the degradation of colelenterazine to coelenteramide and a photon of blue light. Renilla Luciferase is broadly applied as a reporter in *in vitro* cell culture and *in vivo* imaging. GFP (green fluorescent protein) presents green fluorescence, and it was first identified in *Aequorea Victoria*. It has become widely used in cell biology to monitor gene expression, protein localization and interactions. Its popularity prompted the development of mutant variants, such as the eGFP (enhanced GFP). eGFP has a higher intensity emission compared to the GFP molecule. The presence of fluorescent proteins and Renilla Luciferase allows for cell identification and quantification by flow cytometry or fluorescence microscopy, and localization studies *in vivo*, providing easy assay readouts.

Application

- Control in experiments that involve transduction.
- Optimization of transduction assays and tracking transduction efficiency over time.
- Generate Renilla Luciferase-eGFP reporter cell pools or stable cell lines following hygromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of Renilla Luciferase-eGFP Reporter Lentivirus at ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze/thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

Biosafety

The lentiviruses are produced with the SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. 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.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the designed protocol. BPS Bioscience media, reagents, and luciferase assay systems are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
HEK293 Cells	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
TWO-Step Luciferase (Firefly & Renilla) Assay System	BPS Bioscience #60683
96-well white clear-bottom assay plate	Corning #3610
Flow Cytometer or fluorescence microscope Luminometer	

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells. 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with hygromycin prior to carrying out the assays.

Day 1:

1. Harvest HEK293 cells from culture, centrifuge and resuspend the cells in fresh Thaw Medium 1.
2. Count cells.
3. Plate HEK293 cells at a density of 5,000-10,000 cells per well into a clear-bottom 96-well cell culture plate in 100 µl of Thaw Medium 1.
4. Incubate the plate at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 5 µl of Renilla Luciferase-eGFP Lentivirus to the cells.

2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to each well to a final concentration of 5 µg/ml.

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 overnight.

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

Day 3:

1. Remove the medium and add 100 µl of fresh Thaw Medium 1.

   Note: If neither the polybrene nor the lentivirus adversely affect 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.

2. Incubate the plate at 37°C with 5% CO_2 for 24-48 hours.

Day 4-5:

1. The expression of eGFP can be analyzed by microscopy or flow cytometry (Ex/Em=488/510 nm), or another method of interest.

2. To measure Renilla Luciferase activity, use TWO-Step Luciferase (Firefly & Renilla) Assay System (#60683) following the recommended protocol.

Important Notes

To generate a Renilla Luciferase-eGFP expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of hygromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

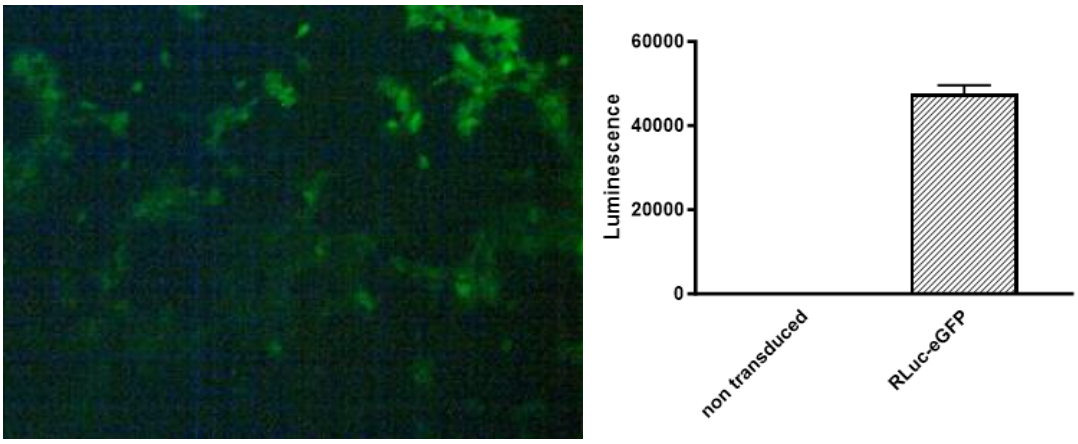

Figure 2. eGFP expression and Renilla Luciferase reporter activity in HEK293 cells transduced with Renilla Luciferase- eGFP Reporter Lentivirus.
Left Panel: Approximately 8,000 HEK293 cells/well were seeded and transduced at the same time with 5 µl/well of Renilla Luciferase-eGFP Lentivirus. 66 hours post-transduction, the expression of eGFP was observed under a fluorescence microscope.
Right Panel: Approximately 8,000 HEK293 cells/well were seeded and transduced at the same time with 5 µl/well of Renilla Luciferase-eGFP Lentivirus. 66 hours post-transduction, Renilla luciferase activity was measured using TWO-Step Luciferase (Firefly & Renilla) Assay System (#60683). Non-transduced cells were run in parallel as negative control.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
eGFP Lentivirus (Inducible TET On)	78629	500 µl x 2
Enhanced GFP Lentivirus (G418)	78639-G	500 µl x 2
Enhanced GFP Lentivirus (Puro)	78639-P	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (EF1a promoter)	78741	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (CMV promoter)	79980	500 µl x 2

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