Description

The Myc Luciferase Reporter 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 a firefly luciferase reporter driven by the Myc response element located upstream of the minimal TATA promoter, and a G418 selection marker (Figure 1). After transduction, the Myc signaling pathway in the target cells can be monitored by measuring the luciferase activity.

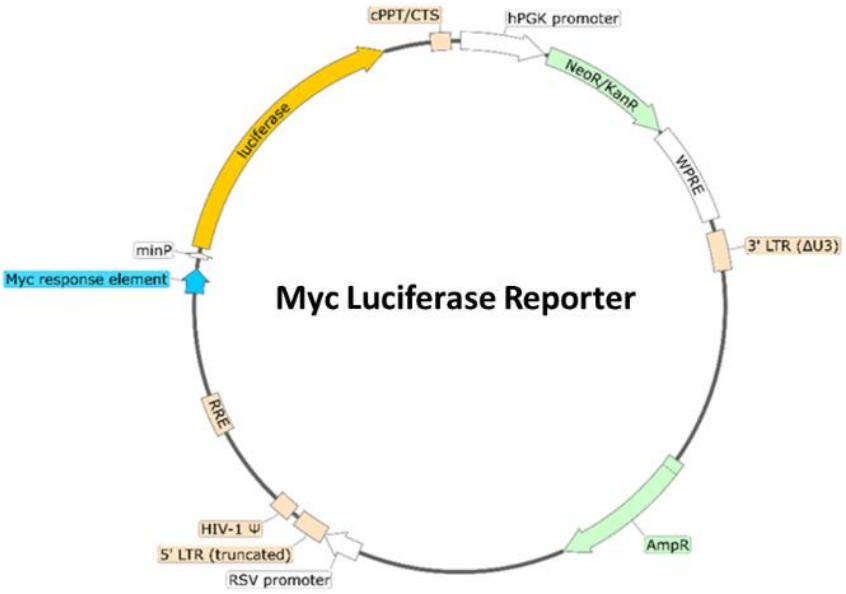

Figure 1: Schematic of the lenti-vector used to generate Myc Luciferase Reporter Lentivirus.

Background

The Myc signaling pathway plays an important role in cell proliferation, differentiation, transformation and apoptosis. c-Myc is a transcription factor that heterodimerizes with MAX to regulate Myc signaling pathway-responsive genes. Myc can be activated by the Wnt/β-catenin pathway. Genetic alterations in MYC have been linked to a number of human cancers, including Burkitt's lymphoma, cervical, ovarian, breast, lung, and pancreatic carcinomas. Thus, Myc is a promising therapeutic target for cancer treatment.

Application(s)

- Screen for activators or inhibitors of the Myc signaling pathway in transduced target cells.
- Generate cell pools or stable cell line expressing the Myc luciferase reporter following G418 selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture 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 lentivirus at a titer >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 virus at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience 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 protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
HCT116 cells	ATCC #CCL-247
ICG-001	Selleckchem #S2662
Thaw Medium 7	BPS Bioscience #60185
Assay Medium 7B	BPS Bioscience #79718
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Negative Control Luciferase Lentivirus	BPS Bioscience #79578
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture, clear-bottom, white plate	Corning #3610
Luminometer	

Media Formulations

For the best results, the use of BPS Bioscience validated and optimized media *is highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 7 (BPS Bioscience #60185):
McCoy's 5A medium with 10% FBS, 1% Penicillin/Streptomycin.

Assay Medium 7B (BPS Bioscience #79718):
Opti-MEM I + 0.5% FBS + 1% non-essential amino acids + 1 mM sodium pyruvate + 1% penicillin/ streptomycin.

Assay Protocol

- HCT116 is a human colon cancer cell line that expresses a mutated β-catenin which leads to the constitutive activation of the downstream Myc transcription factor. Upon transduction with the Myc Luciferase Reporter Lentivirus, the constitutively active β-catenin/Myc axis induces the expression of the Myc luciferase reporter, which can be quantified by measuring luciferase activity.
- The following protocol was used to transduce HCT116 cells. The 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 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 reporter, with G418, prior to carrying out the reporter assays.
- The assay should include "Treated Cells", "Luminescence Background" (no cells) and "Non-Treated Cells" conditions.
- We recommend using Negative Control Luciferase Lentivirus (#78578) as control.

Day 1:

1. Seed cells at a density of 5,000-10,000 cells per well into white, clear bottom 96-well cell culture-treated plates in 90 µl of Thaw Medium 7. Leave a few empty wells as "Luminescence Background".

2. To each well, add 5 µl of Myc Luciferase Reporter Lentivirus.

   Optional: Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer 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 for 48 hours.

Day 3:

1. Remove the medium containing the lentiviruses from the wells.

2. Prepare appropriate dilutions of ICG-001, an β-catenin inhibitor, in Assay Medium 7B (100 µl/well).

3. Add 100 µl of diluted ICG-001 to the "Treated Cells" wells.

4. Add 100 µl of Assay Medium 7B to the "Non-Treated Cells" and "Luminescence Background" wells.

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

Day 4:

1. Add 100 µl of ONE-Step™ reagent to each well.

2. Incubate the plate at room temperature for ~15 to 30 minutes.

3. Measure luminescence using a luminometer.

Validation Data

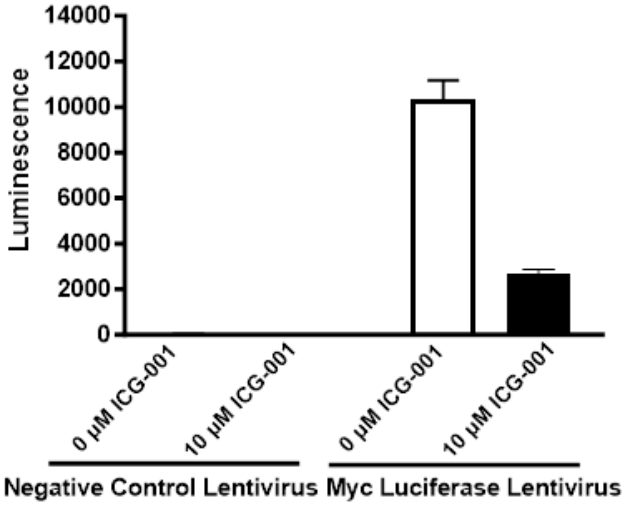

Figure 2. Inhibition of Myc luciferase reporter activity by ICG-001 in HCT116 cells transduced with Myc Luciferase Reporter Lentivirus.
Approximately 10,000 HCT116 cells/well were transduced with 100,000 TU of Myc Luciferase Reporter Lentivirus. 48 hours post-transduction, the medium was changed to Assay Medium 7B or Assay Medium 7B containing 10 µM ICG-001, and the plate was incubated at 37°C with 5% CO_2 overnight. Luciferase activity was measured using ONE-Step™ Assay System. Results are shown as the raw luminescence readings. Negative Control Luciferase Lentivirus (#79578) transduced cells were used in parallel as control.

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

Notes

1. To generate a Myc Luciferase reporter-expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of G418 (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase Lentivirus (#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.
    b. Renilla Luciferase Lentivirus (#79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the control of a CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.
    c. Firefly Luciferase Lentivirus (#79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a CMV promoter. It can serve as a positive control for transduction optimization studies.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

References

Pelengaris S., *et al.*, 2002 *Nat. Rev. Cancer.* 2(10): 764-76.

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*
Myc Reporter Kit (Myc signaling pathway)	60519	500 reactions
Myc Luciferase Reporter Lentivirus (Puromycin)	78628-P	500 µl x 2
Myc Luciferase Reporter HCT116 Cell Line	60520	2 vials
Anti-Myc-Tag Monoclonal	25012	100 µg
Myc Reporter Kit (Myc Signaling Pathway)	60519	500 reactions

Version 041624