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Produktinformation

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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The Myc Luciferase Reporter Lentiviruses 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 gene driven by the Myc response element located upstream of the minimal TATA promoter (Figure 1) and an antibiotic selection gene (puromycin) for the selection of stable clones. After transduction, the Myc signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Application

1. Screen for activators or inhibitors of Myc signaling pathway in transduced target cells
2. Generate Myc Luciferase Reporter stable cell line (puromycin resistant)

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. 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. Myc can be activated by the Wnt/β-catenin pathway.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

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 lentiviruses at -80°C. 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
Polybrene	Millipore, #TR-1003-G
96-well tissue culture, clear-bottom, white plate	Corning, #3610
One-Step luciferase assay system	BPS Bioscience #60690
Luminometer	

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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. HCT116 cells contain a mutated β-catenin which leads to the accumulation of β-catenin and constitutive activation of 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. ICG-001 is an inhibitor of the Wnt/β-catenin pathway.

The following protocol is a general guideline for transducing HCT116 cells using the Myc 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: Seed cells at a density of 5,000-10,000 cells per well into white, clear bottom 96-well microplate in 90 µl of Thaw Medium 7 (BPS Bioscience #60185).

   To each well, add 5 µl of Myc luciferase reporter lentivirus. *Optional: Add polybrene to each well to a final concentration of 5 µg/ml.*

   Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 48 hours.

2. Day 3: Remove the medium containing the lentivirus from the wells.
   Add 100 µl of Assay Medium 7B containing diluted ICG-001 to inhibition wells.

Add 100 µl of Assay Medium 7B to the control untreated wells (to determine the luminescence from the transduced HCT116 cells).

Add 100 µl of Assay Medium 7B to cell-free control wells (for determine the background luminescence).

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

3. Day 4: Perform the ONE-Step™ Luciferase assay (BPS Bioscience #60690) as per recommended protocol (100 µl/well). Incubate the plate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

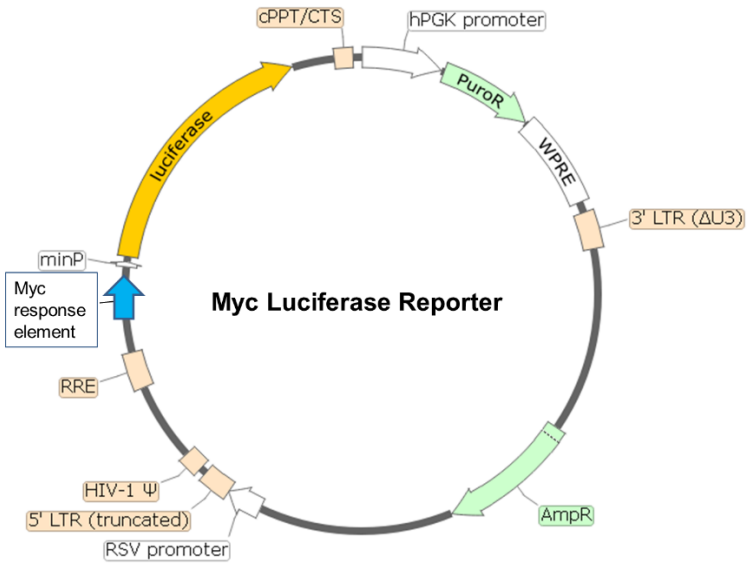

Figure 1. Schematic of the lenti-vector used to generate the Myc luciferase reporter lentivirus.

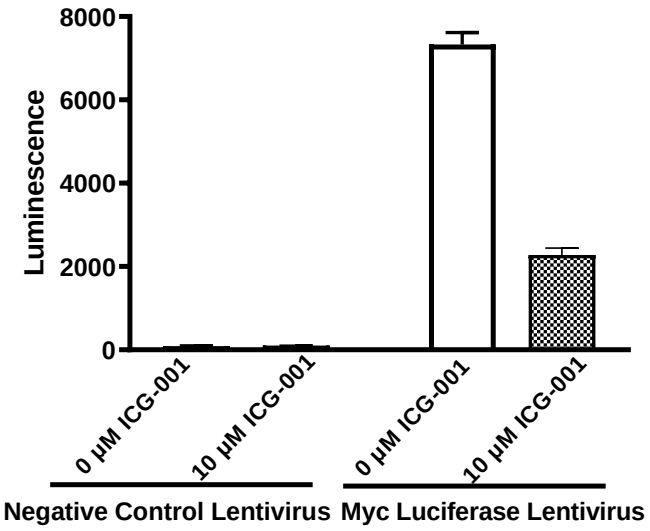

Figure 2. Inhibition of Myc luciferase reporter activity by ICG-001 in HCT116 cells. Approximately 10,000 HCT116 cells/well were transduced with 100,000 TU Myc Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to Assay Medium 7B or Assay Medium 7B containing ICG-001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

Notes

1. To generate the Myc luciferase reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase 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.
    b. Renilla Luciferase 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.
    c. Firefly Luciferase Lentivirus (BPS Bioscience #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. It serves 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) c-MYC: more than just a matter of life and death. *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*
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Myc Reporter (Luc) – HCT116 Recombinant Cell Line (Myc Signaling Pathway)	60520	2 vials
Myc Reporter Kit (Myc Signaling Pathway)	60519	500 reactions
Transfection Collection™: Myc Transient Pack (Myc Signaling Pathway)	79284	500 reactions
NF-κB Reporter (Luc) - HCT116 Recombinant Cell Line	60623	2 vials