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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

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 HSE (Heat Shock Response) Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses transduce cells with the firefly luciferase gene driven by multiple copies of the heat shock response element (HSE) located upstream of the minimal TATA promoter. The lentiviruses also transduce a puromycin selection gene (Figure 1). After transduction, the heat shock response in the target cells can be monitored by measuring luciferase activity.

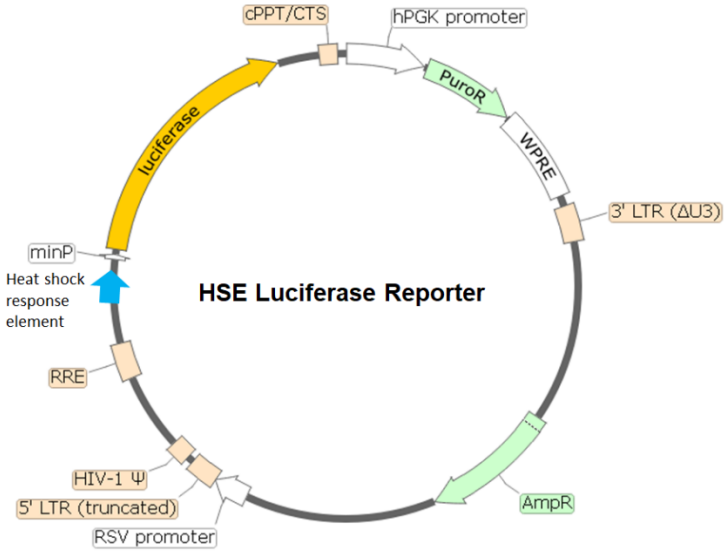

Figure 1. Schematic of the lenti-vector used to generate the HSE luciferase reporter lentivirus.

Background

The Heat Shock Response (HSR) is crucial for cells to adapt to stressful conditions. In the presence of high temperatures, oxidative stress or heavy metals, several proteins can unfold and become unable to perform their normal functions. This in turn can result in cell damage and/or death. To maintain proteins with the proper structure the expression of Heat Shock Proteins (HSP) is induced. Expression of HSPs is regulated by the transcription factor Heat Shock Factor 1 (HSF1). When cellular stress occurs HSF1 undergoes a confirmation change and moves to the nucleus, where it can bind to Heat Shock Elements (HSE) and lead to transcription of the HSP mRNA. Dysfunction in the HSR can lead to pathologies such as neurodegenerative diseases and cancer. Targeting HSE may prove beneficial in the treatment of pathologies linked to defective HSR.

Application

- Expression of HSF1-dependent luciferase reporter to study the heat shock response.
- Generate HSE Luciferase Reporter stable cell lines (puromycin resistant).

Formulation

The lentiviruses were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations 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.

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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 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 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 "Assay Protocol" 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
HeLa Cells	ATCC #CCL-2
Thaw Medium 1	BPS Bioscience #60187
17-Allylaminogeldanamycin (17-AAG)	Sigma #A8476
96-well tissue culture, clear-bottom, white plate	Corning #3610
One-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Media Formulations

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

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience, #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

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Assay Protocol

The following protocol is a general guideline for transducing HeLa cells using HSE Luciferase Reporter Lentiviruses. 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 expressing the reporter gene with puromycin, to generate cell lines, prior to carrying out the reporter assays.

Day 1: Seed HeLa cells at a density of 5,000-10,000 cells per well in 90 µl of Thaw Medium 1 per well of a white, clear bottom 96-well microplate. Add 5 µl of HSE luciferase reporter lentivirus to each well. *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 48-66 hours.

Day 3: Remove the medium containing the lentiviruses from the wells. Add 100 µl of Thaw Medium 1 containing the compound to be tested to the "Test" wells.

Add 100 µl of Thaw Medium 1 to the control "Untreated" wells (to determine the luminescence from the transduced HeLa cells) and to "Cell-free" control wells (to determine the background luminescence).

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

Day 4: Add 100 µl/well of ONE-Step™ Luciferase reagent. Incubate the plate at Room Temperature (RT) for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

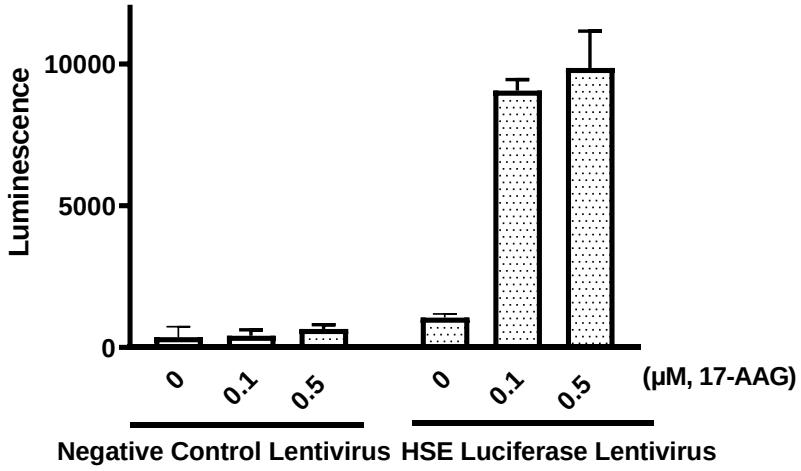

Figure 2. Activation of HSE luciferase reporter activity in HeLa cells by 17-AAG.
Approximately 8,000 HeLa cells/well were transduced with 40,000 TU/well of HSE Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to fresh Thaw Medium 1 containing various concentrations of 17-Allylaminogeldanamycin (17-AAG) (Sigma #A8476), and the plate was incubated at 37°C with 5% CO_2 for 5 hours. Results are shown as the raw luminescence reading. Negative Control Luciferase Lentivirus (BPS Bioscience #79578) were used in parallel as control.

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Notes

1. To generate HSE luciferase reporter stable cells, 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. This negative control is important to establish the specificity of any treatments and to determine background reporter activity.
    b. Renilla Luciferase Lentivirus (BPS Bioscience #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The Renilla luciferase 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

Richter K, *et al*., 2010, *Molecular Cell* 40 (2): 253-266

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
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2

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