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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The ATF6 (Activating Transcription Factor 6) Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce most types of mammalian cells, including primary and non-dividing cells. These viruses transduce cells with the firefly luciferase gene driven by multiple copies of an ATF6 response element, located upstream of the minimal TATA promoter. The lentiviruses also transduce a puromycin selection gene (Figure 1). After transduction, the ATF6-mediated response to ER (endoplasmic reticulum) stress can be monitored by measuring luciferase activity.

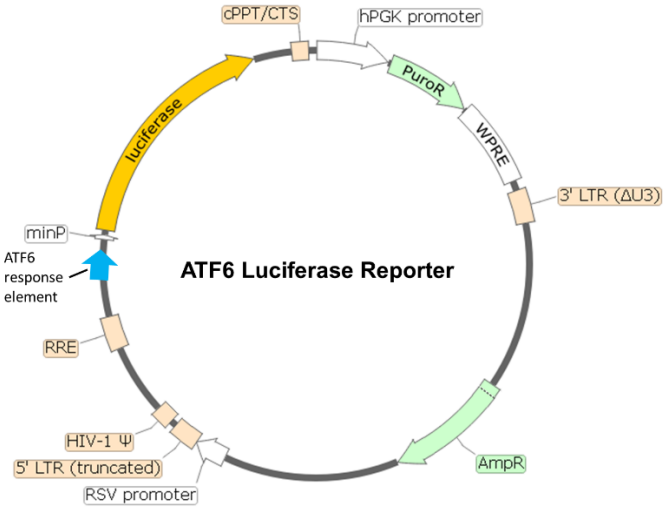

Figure 1. Schematic of the lenti-vector used to generate the ATF6 luciferase reporter lentivirus.

Background

Activating Transcription Factor 6 (ATF6) is a transmembrane transcription factor that responds to endoplasmic reticulum (ER) stress via the unfolded protein response (UPR). In the presence of excess misfolded proteins, the UPR is activated and there is an increase in chaperone expression. ATF6 is one of the three critical proteins of the UPR. In response to ER stress, ATF6 is cleaved, and the cytosolic portion is translocated to the nucleus where it binds to ER stress-response elements on the promoters of target genes, leading to transcription of ER molecular chaperones. Dysfunction in the UPR pathway results in developmental defects, neurodegenerative diseases, and cancer. Strategies targeting ATF6 may prove beneficial for cancer and degenerative disease treatment.

Application

- Expression of ATF6-dependent luciferase reporter to study the ATF6 response pathway.
- Generate ATF6 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
Tunicamycin	Sigma #654380
Clear-bottom, white 96-well tissue culture 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 ATF6 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 create cell lines, prior to carrying out the reporter assays.

Day 1: Seed HeLa cells at a density of 5,000-10,000 cells/well in 90 µl of Thaw Medium 1 into a white, clear bottom 96-well microplate. Add 5 µl of ATF6 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 lentivirus 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).

Add 100 µl of Thaw Medium 1 to "Cell-free" control wells (to determine the background luminescence). Incubate the plate at 37°C with 5% CO_2 overnight.

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

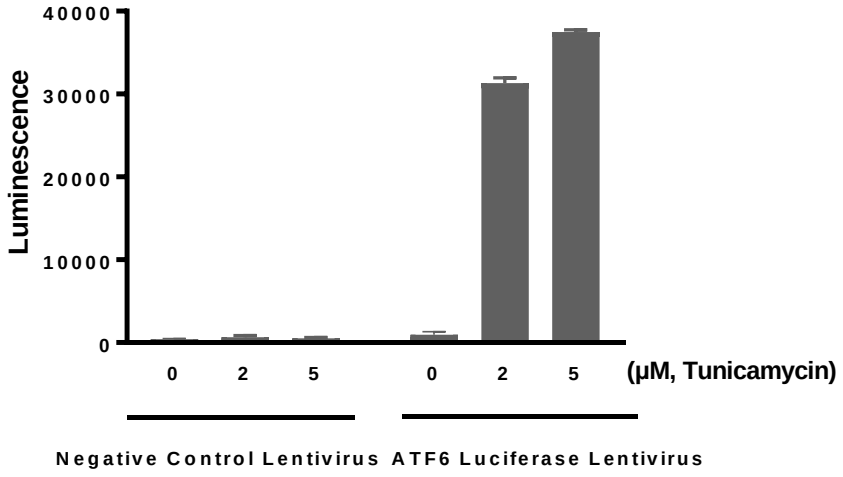

Figure 2. Activation of ATF6 luciferase reporter activity in HeLa cells by tunicamycin.
Approximately 8,000 HeLa cells/well were transduced with 40,000 TU/well of ATF6 Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to fresh Thaw Medium 1 containing various concentrations of tunicamycin (Sigma #654380). The plate was incubated at 37°C with 5% CO_2 for 18 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 ATF6 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

Hillary R. and FitzGeral U., 2018, *Journal of Biomedical Science* 25: 48.

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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