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Produktinformation

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

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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

Estrogen Response Element (ERE) Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses contain a firefly luciferase reporter driven by multiple copies of estrogen response element (ERE) located upstream of the minimal TATA promoter. The lentiviruses also contain a puromycin selection marker (Figure 1). After transduction, the cellular estrogen receptor signaling pathway can be monitored by measuring luciferase activity.

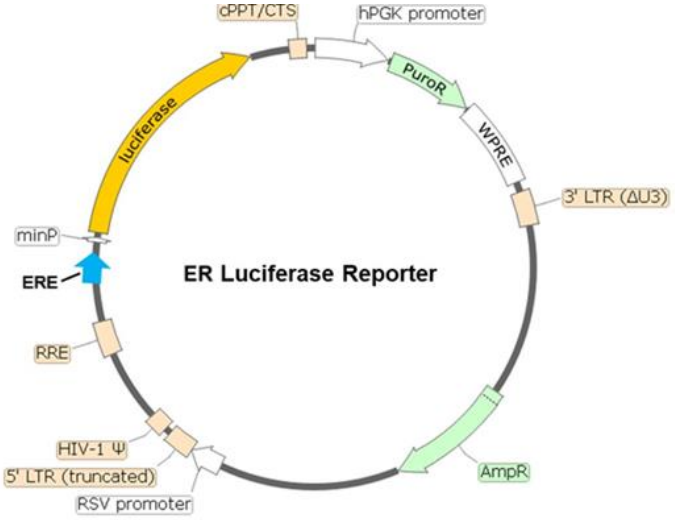

Figure 1. Schematic of the lenti-vector used to generate the Estrogen Response Element (ERE) Luciferase Reporter Lentivirus.

Background

ER (estrogen receptor) exists as two nuclear ER isoforms (ERα and ERβ) and as a membrane associated ER (GPER1), and responds to estrogen. Estrogen is a hormone produced mainly in the ovaries, but also in the adipose tissue, and plays a role in reproduction, bone density, inflammation, and others. Estrogen can penetrate the cell membrane and bind to ERα, which dimerizes and translocates to the nucleus, where it associates with transcriptional co-activators and binds to ERE (estrogen response elements). In addition to this genomic role, ER also plays non genomic roles by directly activating cellular signaling pathways. About 60% of breast cancer (BC) cases are ERα-positive and are classified as luminal BC. Patients with luminal BC have several therapeutic strategies available to them: hormone therapy, SERM (selective estrogen receptor modulators), AI (aromatase inhibitors), SERD (selective estrogen receptor degraders) and LHRH (luteinizing hormone-releasing hormone). However, about half of the metastatic therapy-resistant BC result from ERα mutations, with at least 62 being known. New therapeutic avenues are thus needed for the successful treatment of this BC patient population.

Application

- Screen and characterize modulators of the estrogen receptor signaling pathway.
- Generate estrogen response element luciferase reporter cell pools or stable cell lines following puromycin 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 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 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 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 "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
T47D Cells	ATCC #HTB-133
Assay Medium 2C	BPS Bioscience #78544
Assay Medium 2F	BPS Bioscience #82784
Thaw Medium 2	BPS Bioscience #60184
Insulin Solution	MilliporeSigma #I9278
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 Cell Culture and Functional Cellular Assay

Complete Thaw Medium 2:

Thaw Medium 2 (#60184) + 10 µg/ml Insulin (MilliporeSigma#I9278): RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 10 µg/ml Insulin.

Assay Medium 2C (#78544):
RPMI 1640 (ATCC modification) medium (no phenol red) supplemented with 10% charcoal-stripped FBS and 1% Penicillin and Streptomycin.

Assay Medium 2F (#82784):
RPMI 1640 (ATCC modification) medium (no phenol red) supplemented with 0.5% charcoal-stripped FBS

Assay Protocol

- The following protocol is a general guideline for transducing T47D cells using the Estrogen Response Element (ERE) 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 expressing the reporter gene with puromycin, creating a cell pool or stable cell line, prior to carrying out the reporter assays.
- The assay should include "Stimulated", "Cell-Free Control" and "Control Untreated" conditions.

Day 1:

1. Seed T47D cells at a density of ~3,000 cells per well in 90 µl of Complete Thaw Medium 2 into a white, clear bottom 96-well microplate. Leave a few empty wells as "Cell-Free Control".

2. To each well, add 1 µl of Estrogen Response Element (ERE) Luciferase Reporter Lentivirus.

   Optional: Add polybrene 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 for 24 hours.

Day 2:

1. Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Assay Medium 2C to each well.

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

Day 3:

1. Remove Assay Medium 2C from each well.

2. Add 100 µl of Assay Medium 2F containing the compounds being tested to the "Stimulated" wells.

3. Add 100 µl of Assay Medium 2F to the "Control Untreated" wells (to determine the unstimulated luminescence from the transduced T47D cells).

4. Add 100 µl of Assay Medium 2F to the "Cell-Free Control" wells (to determine the background luminescence).

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

Day 4:

1. Add 100 µl/well of ONE-Step™ Luciferase assay reagent to each well.
2. Incubate the plate at Room Temperature (RT) for ~15 to 30 minutes.
3. Measure luminescence using a luminometer.

Validation Data

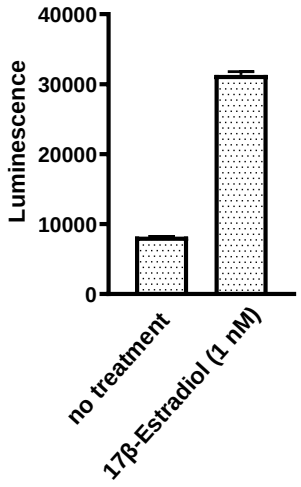

Figure 2. Activation of the estrogen response element (ERE) luciferase reporter activity in T47D cells transduced with Estrogen Response Element (ERE) Luciferase Reporter Lentivirus. Approximately 3,000 T47D cells/well were transduced with 30,000 TU/well of Estrogen Response Element (ERE) Luciferase Reporter Lentivirus. 24 hours post-transduction, cells were switched to Assay Medium 2C and cultured for an extra 24 hours. Cells were then treated with 1 nM of 17β-Estradiol in Assay Medium 2F for 24 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. Results are shown as the raw luminescence reading.

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

Notes

1. To generate an estrogen response element (ERE) 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 (as pre-determined from a killing curve, FAQs (bpsbioscience.com)) 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. This 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 Renilla luciferase lentiviruses 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 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

Juliette L., *et al.*, 1999 *Toxicological Sciences* 48: 55-66.
Yu S., *et al.*, 2017 *Biochemical and Biophysical Research Communications* 486(3): 752-758.
Clusan L., *et al.*, 2023 *Int J Mol Sci* 24(7): 6834.

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
Estrogen Luciferase Reporter T47D Cell Line	82349	2 vials

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