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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

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Description

Androgen Receptor 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 androgen response element (ARE) located upstream of the minimal TATA promoter. The lentiviruses also contain a puromycin selection marker (Figure 1). After transduction, the cellular androgen receptor signaling pathway can be monitored by measuring the luciferase activity.

This product has been validated by testing the response to 5α-Dihydrotestosterone (5-DHT) of the transduced prostate cancer cell lines 22Rv1 and CWR-R1ca, but not LNcaP.

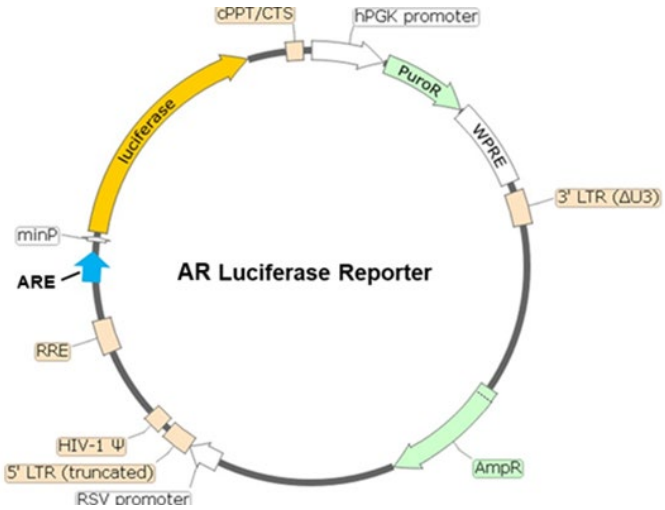

Figure 1. Schematic of the lenti-vector used to generate the Androgen Receptor Luciferase Reporter Lentivirus.

Background

AR (androgen receptor), also known as NR3C4 (nuclear receptor family 3, group C, member 4) is a nuclear receptor involved in regulating gene expression. It is activated by testosterone and dihydrotestosterone, and it has been linked to prostate cancer. Prostate cancer is the most frequently diagnosed cancer and the second-leading cause of cancer death in American men. AR remains functional and is expressed in nearly all primary prostate cancers, with endocrine therapy aiming at reducing serum androgens and inhibiting AR. The androgen-induced transcriptional activation of AR is modulated by the interaction of AR with coregulators and by phosphorylation of AR and AR coregulators in response to growth factors. However, prostate cancer can almost always adapt to survive under castration levels of androgen or to AR inhibition. Castration resistance may involve AR point mutations, overexpression, changes in androgen biosynthesis, expression of constitutively active AR splice variants (active in the absence of ligand binding), and changes in androgen cofactors. As AR activity remains important in the progression of all stages of prostate cancer, AR continues to be an attractive molecular target of drugs against prostate cancer. 22Rv1 cell line is derived from a human prostate cancer xenograph propagated in mice and it is known to form tumors in nude mice. It is mostly dihydrotestosterone-independent for growth but responds to EGF (epidermal growth factor). They express androgen receptor (AR) that responds to androgen stimulation, making it one of the few available cellular models for prostate cancer studies.

Application

- Screen and characterize modulators of the androgen receptor signaling pathway.
- Generate androgen receptor luciferase reporter cell pools or stable cell lines by puromycin selection.

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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
22Rv1 cells	ATCC #CRL-2505
Assay Medium 2C	BPS Bioscience #78544
Thaw Medium 2	BPS Bioscience #60184
5α-Dihydrotestosterone (5-DHT)	Sigma-Aldrich #D-073
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

Thaw Medium 2 (#60184):

RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

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

Assay Protocol

- The following protocol is a general guideline for transducing 22Rv1 cells using the Androgen Receptor 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 22Rv1 cells at a density of ~8,000 cells per well in 90 µl of 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 5 µl of Androgen Receptor 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 48 hours.

Day 3:

1. Prepare the compound of interest at the concentration to be tested, in Assay Medium 2C (100 µl/well).

2. Carefully remove medium from each well.

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

4. Add 100 µl of Assay Medium 2C to the "Control Untreated" wells (to determine the unstimulated luminescence from the transduced 22Rv1 cells).

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

6. Incubate the plate at 37°C with 5% CO_2 for 16-24 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.

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3. Measure luminescence using a luminometer.

Validation Data

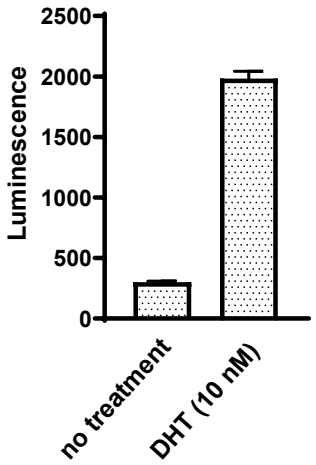

Figure 2. Activation of androgen receptor luciferase reporter activity in 22Rv1 cells transduced with Androgen Receptor Luciferase Reporter Lentivirus.
Approximately 8,000 22Rv1 cells/well were transduced with 80,000 TU/well of Androgen Receptor Luciferase Reporter Lentivirus. 48 hours post-transduction, cells were switched to Assay Medium 2C and stimulated with 10 nM 5α-Dihydrotestosterone (5-DHT) for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase. 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 androgen receptor 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 (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 the background reporter activity.
    b. Renilla Luciferase Lentivirus (BPS Bioscience #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.

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c. Firefly Luciferase Lentivirus (BPS Bioscience #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

Heinlein C.A. and Chang C., 2004 *Endocr Rev.* 25(2):276-308.
Lonergan P.E., and Tindall D.J., 2011 *J Carcinog.* 10:20.
Salami J., *et al.*, 2018 *Commun Biol* 1:100.
Bartonkova I., *et al.*, 2015 PLOS One: 0121316.

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
Androgen Luciferase Reporter 22RV1 Cell Line	78972	2 vials

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