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- Trockeneiszuschlag
- Gefahrgutzuschlag
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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

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

ARE Luciferase Reporter Lentivirus

Catalog #: 79869

Product Description

The Nrf2 antioxidant response pathway plays an important role in the cellular antioxidant defense. Nrf2, a basic leucine zipper transcription factor, induces the expression of antioxidant and phase II enzymes by binding to the ARE (antioxidant response element) region of the gene promoter. Under basal conditions, Nrf2 is retained in the cytosol by binding to the cytoskeletal protein Keap1. Upon exposure to oxidative stress or other ARE activators, Nrf2 is released from Keap1 and translocates to the nucleus, where it can bind to the ARE, leading to the expression of antioxidant and phase II enzymes that protect the cell from oxidative damage.

The ARE Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by ARE located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the Nrf2 antioxidant response pathway in the target cells can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of Nrf2 antioxidant response pathway
- Generation of ARE Luciferase Reporter stable cell line

Formulation

The lentiviruses were produced from HEK293T cells in the medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of ARE luciferase reporter lentivirus at a titer 1 x 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 virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

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Biosafety
The lentiviruses are produced with the third generation 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.

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.

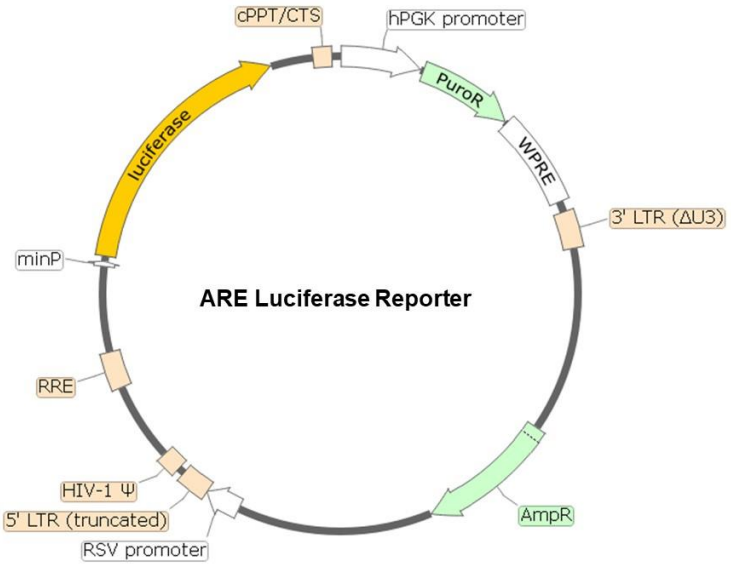

Figure 1. Schematic of the lenti-vector used to generate the ARE luciferase reporter lentivirus

Materials Required but Not Supplied

- DL-Sulforaphane (Sigma, #S4441). Prepare 10 mM stock solution in DMSO.
- HepG2 growth medium or use
  Thaw Medium 9 (BPS Bioscience #79665) (MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01).)
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)
- Luminometer

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Assay Protocol

The following protocol is a general guideline for transducing HepG2 cells using ARE 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: Harvest HepG2 cells from culture and seed cells at a density of 5,000-10,000 cells per well into a white opaque 96-well microplate in 50 µl of HepG2 growth medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of ARE luciferase reporter lentivirus. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.

   Alternatively, seeding cells and the transduction can be performed on the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh HepG2 growth medium to each well.

   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing the medium.

4. Day 4: Prepare diluted DL-Sulforaphane in HepG2 growth medium. Add 10 µl of diluted DL-Sulforaphane to the DL-Sulforaphane-stimulated wells. The final concentration of DMSO in each well should be ≤ 0.1%. Add 10 µl of growth medium to the unstimulated control wells (for measuring the uninduced level of ARE reporter activity).

5. Incubate at 37°C with 5% CO_2 for 16-24 hours.

6. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes:

1. To generate the ARE luciferase reporter stable cell line, on day 4 remove HepG2 growth medium and replaced it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

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2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

1) Negative Control 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.

2) Renilla Luciferase (Rluc) 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.

3) Firefly Luciferase (Fluc) Lentivirus (BPS Bioscience, #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. The Fluc lentivirus can serve as a positive control for transduction optimization studies.

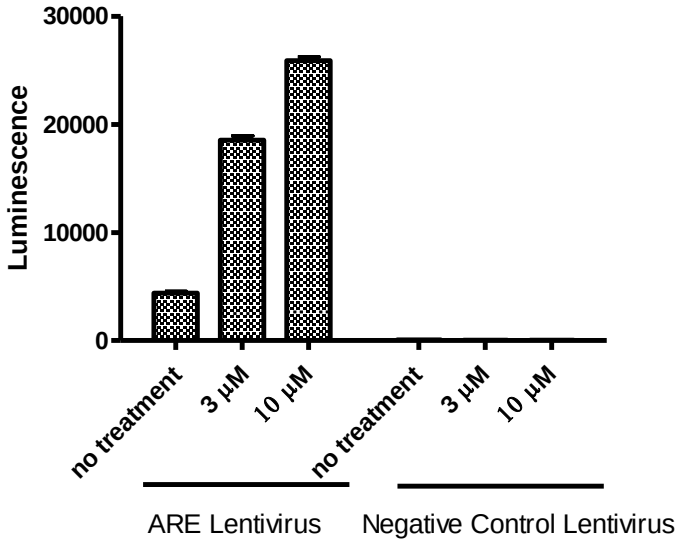

Figure 2. ARE luciferase reporter activity stimulated by DL-Sulforaphane in HepG2 cells. Approximately 10,000 HepG2 cells/well were transduced with 100,000 TU/well ARE luciferase reporter lentivirus. After 48 hours of transduction, medium was changed to HepG2 growth medium, and the cells were treated with DL-Sulforaphane (3 µM or 10 µM) for 18 hours. The results are shown as the raw Luminescence reading.

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Related Products

Product	Cat. #	Size
ARE Reporter HepG2 Cell Line	60513	2 vials
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
ARE Reporter Kit (Nrf2 Antioxidant Pathway)	60514	500 rxns.
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml