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Data Sheet

AP1 Luciferase Reporter Lentivirus (JNK Signaling Pathway)

Catalog #: 79823

Product Description

The stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) family of proteins includes mitogen-activated protein kinases (MAPKs) that are activated by stress, inflammatory cytokines, mitogens, oncogenes, and inducers of cell differentiation and morphogenesis. Upon activation of the SAPK/JNK pathway, MAP Kinase Kinases phosphorylate and activate JNKs. The activated JNKs translocate to the nucleus where they phosphorylate and activate transcription factors such as c-Jun. c-Jun then binds to the activator protein-1 (AP1) response element and induces AP1 transcription.

The AP1 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 AP1 response element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the JNK signaling pathway and AP1 mediated activity in the target cells can be monitored by measuring the luciferase activity.

Applications

- Screen for activators or inhibitors of the JNK signaling pathway in the transduced target cells
- Generation of AP1 Luciferase Reporter stable cell line

Formulation

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

Titer

Two vials (500 µl x 2) of AP1 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 recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

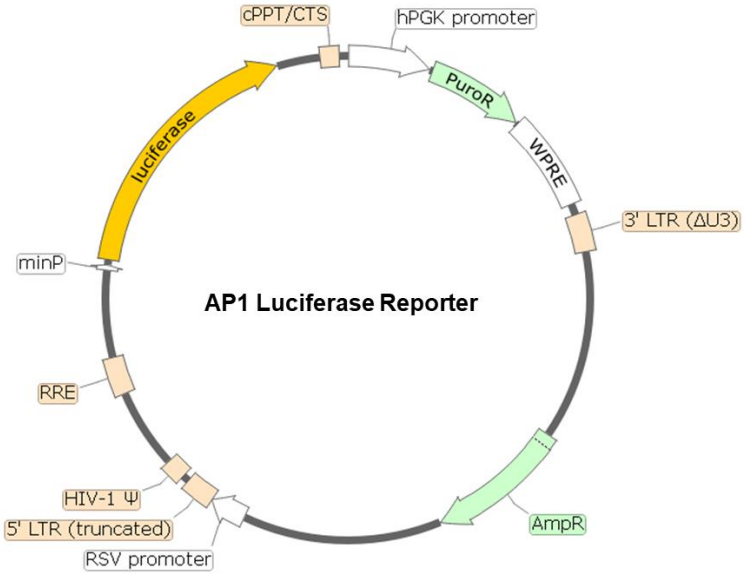

Figure 1. Schematic of the lenti-vector used to generate the AP1 luciferase reporter lentivirus

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Materials Required but Not Supplied

- Phorbol 12-Myristate 13-Acetate (PMA) (LC Laboratories, #P-1680). Prepare stock solution in DMSO.
- HEK293 growth medium or use Thaw Medium 9 (BPS Bioscience, #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- Assay medium: Assay Medium 1B (BPS Bioscience, #79617): Opti-MEM I (Life technologies, #31985062), 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Pen/Strep
- 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

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using AP1 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 HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 9 (BPS Bioscience #79665). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of AP1 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 at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Thaw Medium 9 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 medium.

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4. On the morning of Day 5, carefully remove the medium from the wells, and add 90 µl of assay medium into each well. Prepare diluted PMA in assay medium. Add 10 µl of diluted PMA to the PMA-stimulated wells. Add 10 µl of assay medium to the unstimulated control wells (for measuring the uninduced level of AP1 reporter activity). The final concentration of DMSO in each well is 0.1%.

5. Incubate at 37°C with 5% CO_2 for 5-6 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 AP1 luciferase reporter stable cell line, on day 4 remove Thaw Medium 9 (BPS Bioscience, #79665) and replaced it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

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.

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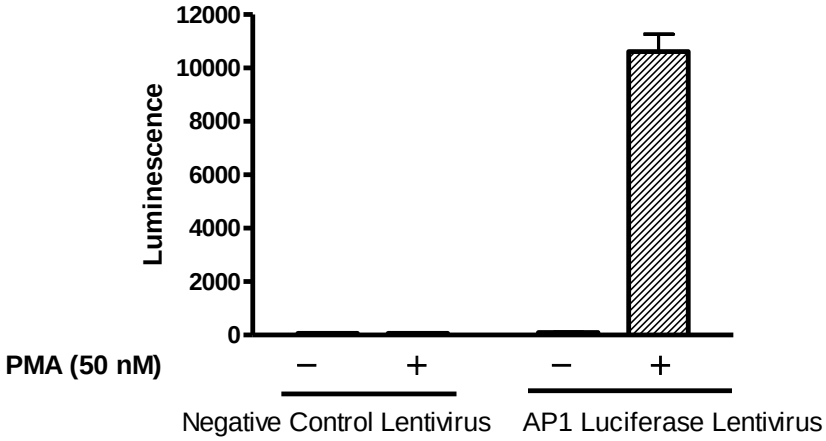

Figure 2. AP1 luciferase reporter activity stimulated by PMA in HEK293 cells. Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well AP1 luciferase reporter lentivirus. After 66 hours of transduction, medium was changed to assay medium, and the cells were treated with 50 nM PMA for ~5 hours. The negative control lentivirus was used in parallel as controls. The results are shown as the raw luciferase reading.

Related Products

Product	Cat. #	Size
NF-κB 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
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	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
AP1 Reporter Kit (JNK Signaling Pathway)	60612	500 rxns
AP1 Reporter (Luc) - HEK293 Cell line	60405	2 vials

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ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml