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Data Sheet

TEAD Luciferase Reporter Lentivirus (Hippo Pathway)

Catalog #: 79833

Product Description

The Hippo pathway regulates cell proliferation and cell death. It is activated by high cell density and cell stress to stop cell proliferation and induce apoptosis. The mammalian Hippo pathway comprises MST kinases and LATS kinases. When the Hippo pathway is activated, MST kinases phosphorylate LATS kinases, which phosphorylate transcriptional co-activators YAP and TAZ. Unphosphorylated YAP and TAZ remain in nucleus and interact with TEAD/TEF transcriptional factors to turn on cell cycle-promoting gene transcription. However, when phosphorylated, YAP and TAZ are recruited from the nucleus to the cytosol, so that the YAP and TAZ-dependent gene transcription is turned off. Dysfunction of the Hippo pathway is frequently detected in human cancer and its down-regulation correlates with the aggressive properties of cancer cells and poor prognosis.

The TEAD 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 the TEAD response elements located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the Hippo pathway in the target cells can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of the Hippo pathway in the transduced target cells
- Generation of TEAD 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 TEAD 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.

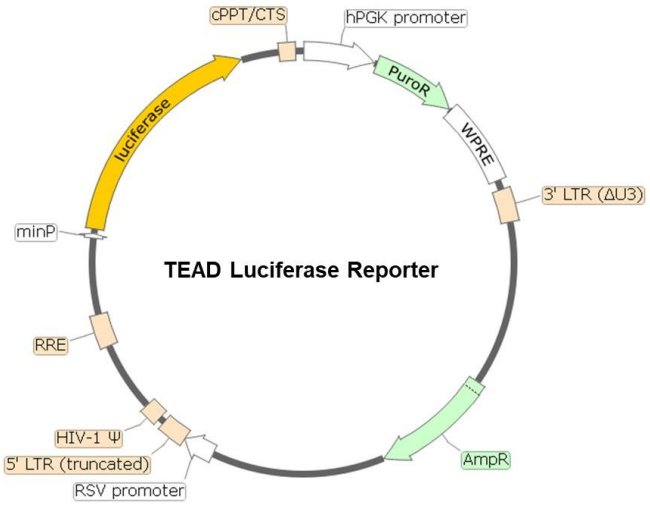

Figure 1. Schematic of the lenti-vector used to generate the TEAD luciferase reporter lentivirus

Materials Required but Not Supplied

- H_2O_2 (Fisher Scientific # H323-500): activator of Hippo pathway
- Okadaic acid (BPS Bioscience #27047): prepare 10 mM stock in DMSO.
- Insulin Solution from Bovine Pancreas (Sigma-Aldrich #: I0516)
- Assay Medium: Thaw Medium 1 (BPS Bioscience #60187) + 10 µg/ml of insulin
- 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 MCF7 cells using TEAD 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 requirement. 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 MCF7 cells from culture and seed cells at a density of 10,000 cells per well into white opaque 96-well microplate in 50 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of TEAD 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 assay 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 medium.

4. On the morning of Day 5, prepare diluted Okadaic acid or H_2O_2 in assay medium. Add 10 µl of diluted Okadaic acid or H_2O_2 to the stimulated wells. The final concentration of DMSO in assay medium is 0.1%. Add 10 µl of assay medium with the same concentration of DMSO without activators to the control wells (for measuring the uninduced level of TEAD reporter activity).

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.

<u>Important Notes:</u>

1. To generate the TEAD luciferase reporter stable cell line, on day 4 remove the medium and replaced it with fresh 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.

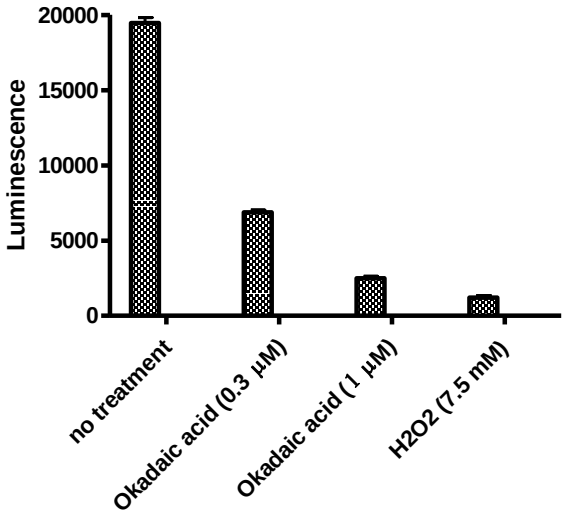

Figure 2. TEAD luciferase reporter activity stimulated by Okadaic acid or H_2O_2 in MCF7 cells. Appropriate 10,000 MCF7 cells/well were transduced with 100,000 TU/well TEAD luciferase reporter lentivirus. After 48 hours of transduction, medium was changed to fresh assay medium, and the cells were treated with Okadaic acid or H_2O_2 for ~ 6 hours. The results are shown as raw luminescence reading.

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Related Products

Product	Cat. #	Size
TEAD Reporter-MCF7 Cell Line	60618	2 vials
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
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
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml