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Data Sheet

Hippo Pathway TEAD Reporter – MCF7 Cell Line Catalog #: 60618

Background

The Hippo pathway regulates cell proliferation and cell death. It is activated by high cell density and cellular stress to stop cell proliferation and induce apoptosis. The mammalian Hippo pathway comprises of the MST and LATS kinases. When the Hippo pathway is activated, MST kinases phosphorylate the LATS kinases, which phosphorylate the transcriptional co-activators YAP and TAZ. Dephosphorylated YAP and TAZ remain in the nucleus and interact with the TEAD transcriptional factors to induce cell cycle-promoting gene transcription. However, when phosphorylated, YAP and TAZ are sequestered in the cytosol, and 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.

Description

The TEAD Reporter – MCF7 cell line contains the firefly luciferase gene under the control of TEAD responsive elements stably integrated into the human breast cancer cell line MCF7. Inside the cells, basal dephosphorylated YAP and TAZ remain in the nucleus and induce constitutive expression of the luciferase reporter. The cell line is validated for the inhibition of the expression of the luciferase reporter by the activators of the Hippo pathway, as well as activation of the luciferase reporter by inhibitors of the Hippo pathway.

Application

- Monitor Hippo pathway activity.
- Screen for activators or inhibitors of the Hippo pathway.

Format

Each vial contains $\sim 1.5 \times 10^6$ cells in 1 ml of 10% DMSO.

Storage

Store in liquid nitrogen immediately upon receipt.

General Culture Conditions

Thaw Medium 1 (BPS Bioscience #60187) + 10 μ g/ml of Insulin (Sigma-Aldrich #I0516): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), plus 10 μ g/ml of insulin (Sigma-Aldrich # I0516).

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Growth Medium 1B (BPS Bioscience #79531) + 10 µg/ml of Insulin (Sigma-Aldrich #I0516): Thaw Medium 1 (BPS Cat. #60187) + 10 µg/ml of insulin (Sigma-Aldrich #I0516), and 400 µg/ml of Geneticin (Invitrogen #11811031).

Cells should be grown at 37°C with 5% CO₂ using Growth Medium 1B with 10 µg/ml of Insulin. It may be necessary to adjust the percentage of CO₂ in the incubator depending on the NaHCO₃ level in the basal medium.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 + Insulin (**no Geneticin**), spin the cells down, resuspend the cells in pre-warmed Thaw Medium 1 + Insulin (**no Geneticin**), and then transfer the resuspended cells to a T25 flask and culture in a CO₂ incubator at 37°C overnight. The next day, replace the medium with fresh Thaw Medium 1 + Insulin (**no Geneticin**), and continue growing culture in a CO₂ incubator at 37°C until the cells are ready to be split. At the first passage, switch to Growth Medium 1B + 10 µg/ml of Insulin (**includes Thaw Medium 1, Insulin, and Geneticin**). Cells should be split before they reach complete confluence.

To passage the cells, rinse the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA. Add Growth Medium 1B + 10 µg/ml of Insulin (Includes Thaw Medium 1, Insulin, and Geneticin) and transfer to a tube, spin down the cells, and then resuspend the cells and seed appropriate aliquots of the cell suspension into new culture vessels. Sub-cultivation ratio: 1:5 to 1:10 weekly.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with Trypsin/EDTA. Add Growth Medium 1B + 10 µg/ml of Insulin (Includes Thaw Medium 1, Insulin, and Geneticin) and transfer to a tube, spin the cells down, and then resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly into the liquid nitrogen.

Functional Validation and Assay Performance

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied for Cell Culture

- Thaw Medium 1 (BPS Bioscience #60187) + 10 µg/ml of insulin
- Growth Medium 1B (BPS Bioscience #79531) + 10 µg/ml of insulin
- Insulin Solution from Bovine Pancreas (Sigma-Aldrich #I0516)

Materials Required but Not Supplied for Cellular Assay

- H₂O₂: activator of Hippo pathway (activate MST kinases)
- Insulin
- Assay Medium: Thaw Medium 1 (BPS Bioscience #60187) + 10 µg/ml of insulin
- Insulin Solution from Bovine Pancreas (Sigma-Aldrich #I0516)

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- Okadaic acid (BPS bioscience #27047): activator of Hippo pathway (activate MST kinases). Prepare 10 mM stock in DMSO.
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Bioscience #60690)
- Luminometer

Mycoplasma testing

The cell line has been screened using the PCR-based VenorGeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Inhibition of TEAD reporter activity by activator of Hippo pathway in TEAD Reporter – MCF7 cells

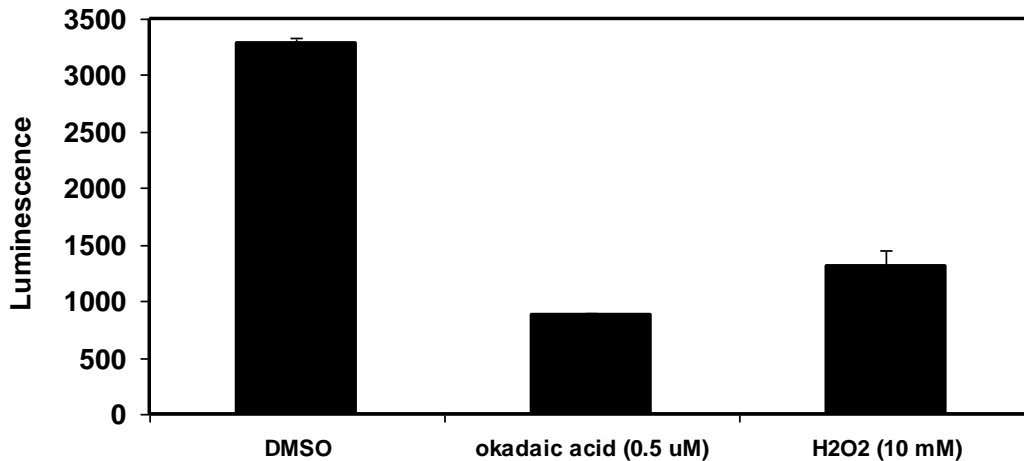
- 1) Harvest TEAD Reporter – MCF7 cells from culture in growth medium and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 45 µl of assay medium.
- 2) Incubate cells at 37°C in a CO₂ incubator for overnight.
- 3) Dilute the activators (H₂O₂ or okadaic acid) stock in assay medium. Add 5 µl of diluted activators to the wells. The final concentration of DMSO in assay medium is 0.1%.
- 4) Add 5 µl of assay medium with same concentration of DMSO without activator to control wells.
- 5) Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).
- 6) Set up each treatment in at least triplicate.
- 7) Incubate cells at 37°C in a CO₂ incubator for 5-6 hours.
- 8) Perform luciferase assay using the ONE-Step™ Luciferase Assay System following the protocol provided: Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. *Note: If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.*
- 9) Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

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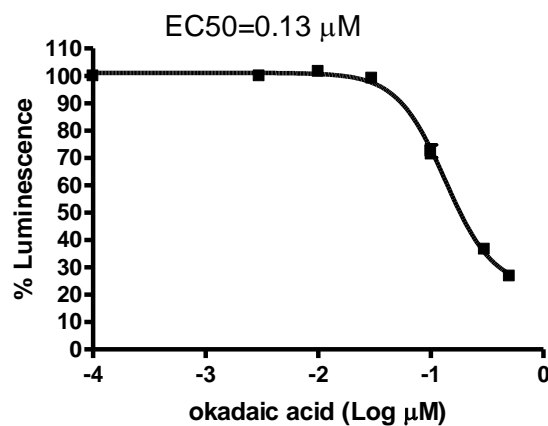
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Figure 1. Response of TEAD Reporter – MCF7 cells to Hippo pathway activators.

1A. H₂O₂ and Okadaic Acid Blocked TEAD Reporter Activity. The results are shown as background-subtracted luminescence values.



1B. Okadaic Acid Inhibition Dose Response Curve. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of okadaic acid was set at 100%. The EC₅₀ of okadaic acid is ~ 0.13 μ M.



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1C. H₂O₂ Inhibition Dose Response Curve. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of H₂O₂ was set at 100%. The IC₅₀ of H₂O₂ is ~ 7.5 mM.

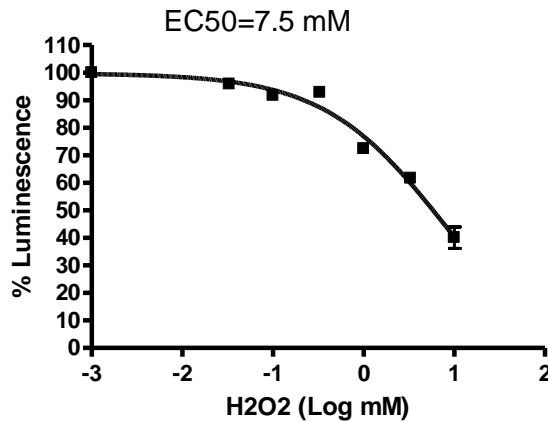
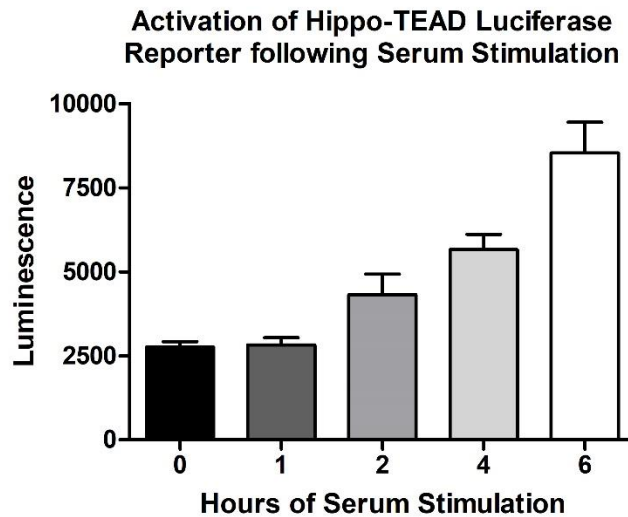


Figure 2. Response of TEAD Reporter – MCF7 cells to Hippo pathway inactivators. TEAD Reporter – MCF7 cells were seeded at a density of 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of assay medium. Cells were then incubated at 37°C in a CO₂ incubator for overnight. The next day, the assay medium was removed and 100 µl of **Thaw medium 1 without serum** was added to each well to serum starve the cells. Cells were then incubated at 37°C in a CO₂ incubator for overnight. The next day, 100 µl of assay medium was added to wells in triplicate for the indicated times. Luciferase was measured using the ONE-Step™ Luciferase Assay System.



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2. Hata Y *et al.* (2013) Okadaic Acid: a tool to study the hippo pathway. [*Mar Drugs.* 11\(3\):896-902.](#) doi: 10.3390/md11030896.
3. Bao Y *et al.* (2011) A cell-based assay to screen stimulators of the Hippo pathway reveals the inhibitory effect of dobutamine on the YAP-dependent gene transcription. [*J Biochem.* 150\(2\):199-208.](#)

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