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 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.

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 unphosphorylated YAP/TAZ remains in the nucleus and induces the constitutive expression of luciferase reporter. The cell line is validated for the inhibition of the expression of luciferase reporter by the activators of the Hippo pathway.

Application

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

Format

Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

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.

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Materials Required but Not Supplied

- H_2O_2: activator of Hippo pathway (activate MST kinases)
- Okadaic acid (AMS bio # 27047): activator of Hippo pathway (activate MST kinases). Prepare 10 mM stock in DMSO.
- Assay medium: growth medium without Geneticin.
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- Luciferase reagents for measuring firefly luciferase activity (We use ONE-Glo luciferase assay system, Promega # E6110. Other luciferase assay systems are also suitable).
- Luminometer

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 CO_2 incubator for overnight.

3) Dilute the activators (H_2O_2 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 CO_2 incubator for 5-6 hours.

8) Perform luciferase assay using the ONE-Glo luciferase assay system: Add 50 μl of One-Glo 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.

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

1A. H_2O_2 and Okadaic Acid Blocked TEAD Reporter Activity.
The results are shown as background-subtracted luminescence values.
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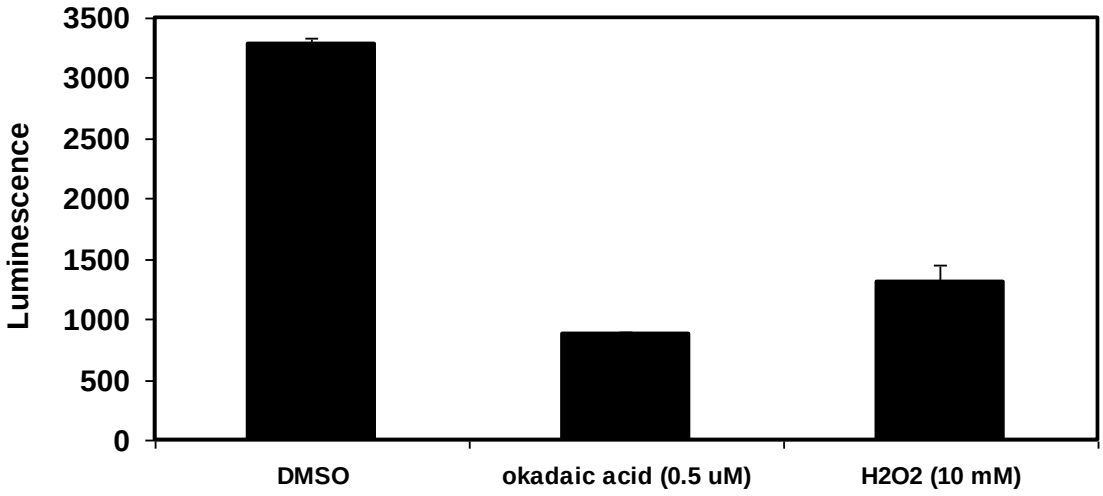
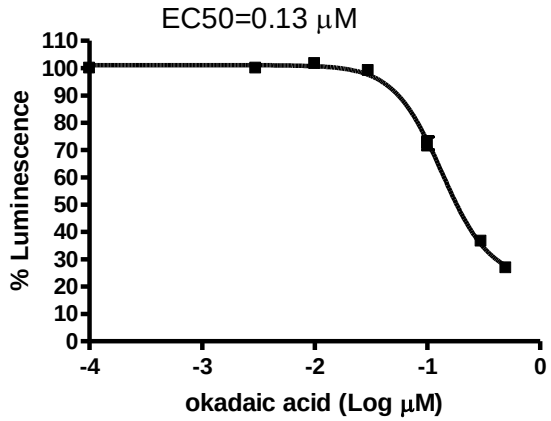
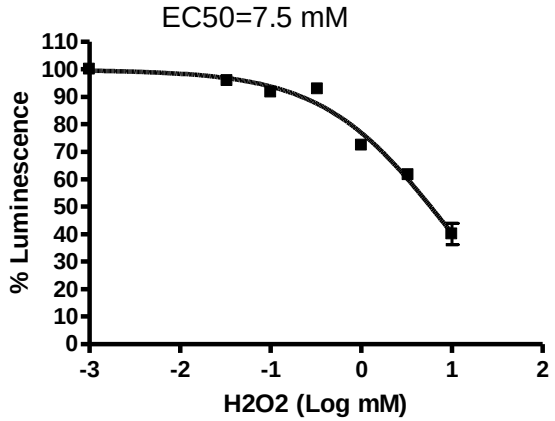


1B. Okadaic Acid Inhibition Dose Response Curve
The results are shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of okadaic acid was set at 100%. The EC50 of okadaic acid is ~ 0.13 μM.



1C. H2O2 Inhibition Dose Response Curve
The results are shown as percentage of luminescence. The background-subtracted luminescence of cells in the absence of H_2O_2 was set at 100%. The IC50 of H2O2 is ~ 7.5 mM.



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.

Storage
Immediately upon receipt, store in liquid nitrogen.

Culture conditions
Cells should be grown at 37° with 5% CO_2 using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acid (Hyclone #SH30238.01), 1mM Na-pyruvate (Hyclone #SH30239.01), 10 μg/ml of insulin, 1% Penicillin/Streptomycin (Hyclone SV30010.01), plus 400 μg/ml of Geneticin (invitrogen #11811031). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

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 growth medium without Geneticin, spin down cells, resuspend cells in pre-warmed growth medium without Geneticin, transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C overnight. The next day, replace the growth medium with fresh growth medium without Geneticin, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. At first passage, switch to growth medium containing Geneticin. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.25% Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down the cells, then, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:5 to 1:10 weekly.

References

Lamar JM *et al.* (2012) The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. *Proc Natl Acad Sci U S A.* 109(37):E2441-50. doi: 10.1073/pnas.1212021109.

Hata Y *et al.* (2013) Okadaic Acid: a tool to study the hippo pathway. *Mar Drugs.* 11(3):896-902. doi: 10.3390/md11030896.

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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