Description

The TEAD Luciferase 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. When the Hippo pathway is not stimulated, transcriptional coactivators YAP (Yes-associated protein) and TAZ (also known as WWTR1, WW domain containing transcription regulator 1) are localized in the nucleus and induce constitutive expression of the luciferase reporter. The cell line has been validated for the inhibition of the luciferase reporter by activators of the Hippo pathway, and for the activation of the luciferase reporter by inhibitors of the Hippo pathway.

Background

The Hippo cell signaling pathway regulates organ size and acts as a tumor suppressor by suppressing the transcription of genes controlled by transcription factor TEAD (Transcriptional enhancer factor TEF-1). The Hippo pathway is activated by high cell density and cellular stress to stop cell proliferation and induce apoptosis. Activation of this pathway triggers a kinase cascade in which MST (Macrophage-stimulating) kinases phosphorylate LATS (Large tumor suppressor) kinases, which then phosphorylate transcriptional co-activators YAP and TAZ. In unstimulated cells, dephosphorylated YAP and TAZ are localized in the nucleus and interact with TEAD transcriptional factors to induce cell cycle-promoting gene transcription. Upon stimulation of the Hippo pathway, newly phosphorylated YAP and TAZ are sequestered in the cytosol, and TEAD-dependent gene transcription is turned off. Dysfunction of the Hippo pathway is frequently detected in human cancer and correlates with cancer aggressiveness and poor prognosis.

Applications

- Monitor Hippo signaling pathway
- Screen for activators or inhibitors of the Hippo pathway
- Measure the activity of transcription factor TEAD

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

MCF7 cells, human breast mammary gland cell line, epithelial cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531
Insulin Solution from Bovine Pancreas	Sigma-Aldrich #I0516

Materials Used in the Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Insulin Solution from Bovine Pancreas	Sigma-Aldrich #I0516
Okadaic acid (stock 10 mM in DMSO)	BPS Bioscience #27047
H_2O_2	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white clear-bottom assay plate	
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

These cells require insulin (10 µg/ml) for optimal growth. Add insulin to the media for cell growth and for cellular assays.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

*Growth Medium 1B (BPS Bioscience #*79531*)* ***plus insulin****:*
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml Geneticin and **10 µg/ml insulin.**

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187) plus insulin:
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and **10 µg/ml insulin.**

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1 **(no Geneticin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin)**.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 **(no Geneticin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1B **(contains Geneticin and insulin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1B and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1B **(contains Geneticin and insulin)**. Seed into new culture vessels at the desired sub-cultivation ratio.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1B and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for storage.


Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

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

The experiment should be performed in triplicates.

1. Seed TEAD reporter MCF7 cells at a density of 35,000 cells/well into a white clear-bottom 96-well cell culture plate, in 45 µl of Thaw Medium 1 containing insulin. Keep 3 wells without cells to determine the background luminescence.
2. Incubate the cells at 37°C in a CO_2 incubator overnight.

3. Prepare the test activators (for example H_2O_2 or okadaic acid) at a concentration 10-fold higher than the desired final concentration by diluting the stock activator solution in Thaw Medium 1 containing insulin. Prepare enough to add 5 µl/well; the final volume will be 50 µl/well.
4. Add 5 µl of diluted activators to the wells. The final concentration of DMSO in the Thaw Medium should not exceed 0.1%.
5. Add 5 µl of Thaw Medium 1 (with insulin) with the same concentration of DMSO as used with the activator to "untreated control" wells.
6. Add 50 µl of Thaw Medium 1 (with insulin) with the same concentration of DMSO as used with the activator to cell-free control wells (for determining background luminescence).
7. Incubate the cells at 37°C in a CO_2 incubator for 5-6 hours.
8. Perform the 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 before reading the luminescence.
9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

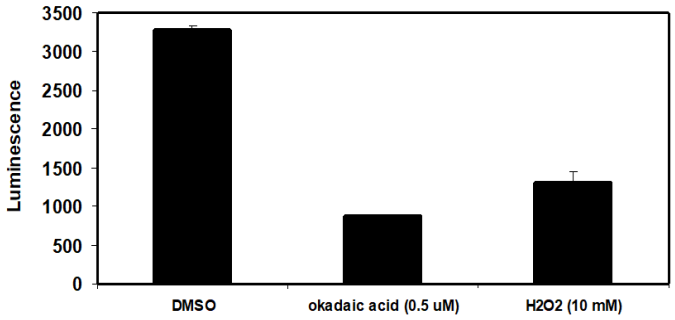

Figure 1: Inhibition of TEAD activity in response to H_2O_2 and Okadaic Acid. TEAD Reporter MCF7 cells were treated with 0.5 µM okadaic acid or 10 mM H_2O_2 as described in the protocol above. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Raw luminescence signal (background-subtracted) is shown.

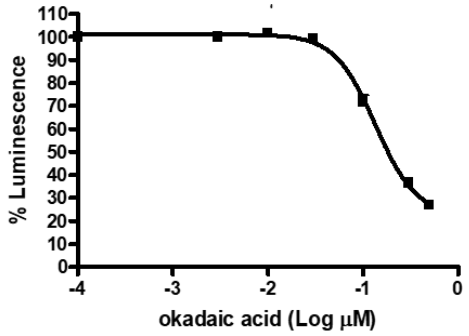

Figure 2: Inhibition of TEAD activity in response to Okadaic Acid. TEAD Reporter MCF7 cells were treated with increasing doses of okadaic acid for 5-6 hours. Luciferase activity was determined using the ONE-Step Luciferase Assay System (BPS Bioscience #60690). Results are expressed as percent of untreated control cells (set at 100%). The EC_{50} of okadaic acid is ~ 0.13 µM.

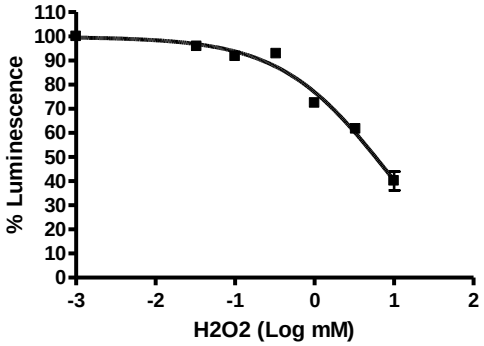

Figure 3: Inhibition of TEAD activity in response to H_2O_2. TEAD Reporter MCF7 cells were treated with increasing doses of H_2O_2 for 5-6 hours. Luciferase activity was measured using the ONE-Step Luciferase Assay System (BPS Bioscience #60690). Results are expressed as percent of untreated control cells (set at 100%). The EC_{50} of H_2O_2 is ~ 7.5 mM.

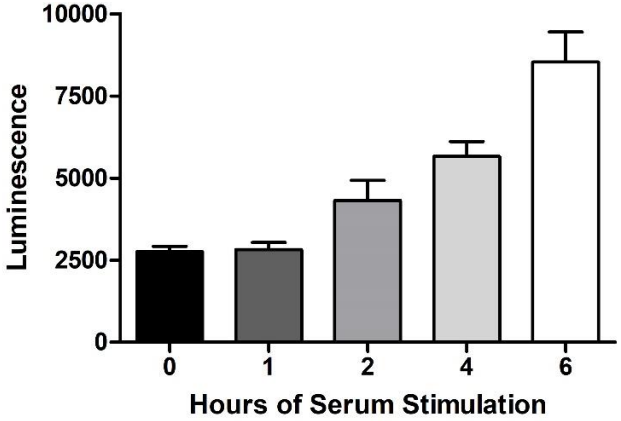

Figure 4: Activation of TEAD in response to Serum. TEAD Reporter MCF7 cells were seeded at 35,000 cells/well the day before the experiment. On the day of the experiment, the culture medium was removed and 100 µl of MEM

medium supplemented with 1% non-essential amino acids, 1 mM Na pyruvate, and 1% Penicillin/Streptomycin (no Serum or insulin) was added. Cells were incubated at 37°C in a CO_2 incubator overnight. The next day, 100 µl of Thaw Medium 1 (containing 10% Serum) was added to cells for the indicated times. Luciferase activity was determined using the ONE-Step Luciferase Assay System (BPS Bioscience #60690).

References

Lamar JM *et al.* (2012) The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. *Proc Natl Acad Sci USA*. **109(37)**: E2441-50.

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

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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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TEAD Luciferase Reporter Lentivirus	79833	500 µl x 2
CRISPRa (SAM) MCF7 Cell Line	78522	2 vials
Cas9-Expressing MCF7 Cell Pool	78179	2 vials