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 Reporter – HEK293 cell line contains a firefly luciferase gene under the control of AP1-responsive elements that are stably integrated into HEK293 cells. This cell line is validated for its response to stimulation by Phorbol 12-Myristate 13-Acetate (PMA) and to treatment with inhibitors of the JNK signaling pathway.

Application

- Monitor the JNK signaling pathway activity and AP1-mediated activity.
- Screen for compound activity of the JNK signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~1.5 x 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Host Cell

HEK293

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.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Materials Required for Cellular Assay

Name	Ordering Information
Phorbol 12-Myristate 13-Acetate (PMA)*	Sigma-Aldrich #P-8139
CC-401 (JNK-401)**	Sigma-Aldrich #SML-1613
Assay Medium 1B	BPS Bioscience #79617
96-well tissue culture-treated white clear-bottom assay plate	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

*Prepare stock solution in DMSO
**Prepare stock solution in water

Storage Conditions

Cells will arrive upon 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. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does not contain selective antibiotics. However, Growth Media does contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO2 using Growth Medium 1B.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 1B (BPS Bioscience #79531):
Thaw Medium 1 (BPS Bioscience Cat. #60187) and 400 µg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO2 using Growth Medium 1B (BPS Bioscience #79531).

Media Required for Cellular Assay

Assay Medium 1B (BPS Bioscience #79617):
Opti-MEM I (Life Technologies # 31985-062), 0.5% FBS, 1% non-essential amino acids, 1mM Na pyruvate, and 1% Pen/Strep

Cell Culture Protocol
To thaw the cells:

1. 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 (no Geneticin).
2. Spin down cells, resuspend cells in pre-warmed Thaw Medium 1 (no Geneticin), transfer resuspended cells to a T25 flask and culture in a CO2 incubator at 37°C. At first passage, switch to Growth Medium 1B (contains Thaw Medium 1 and Geneticin).
3. Cells should be split before they reach complete confluence.

To passage the cells:

1. Rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel using 0.05% Trypsin/EDTA, add Growth Medium 1B transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.
2. Subcultivation ration: 1:10 to 1:20 weekly.

To freeze down the cells:

1. Rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA.
2. Add Growth Medium 1B and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS).
3. Place at -80°C overnight and place in liquid nitrogen the next day.

Assay Protocol

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

A. Response of AP1 Reporter – HEK293 cells to PMA

1. Harvest AP1 Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 1.

2. Incubate cells at 37°C in a 5% CO2 incubator overnight.

3. The next day, carefully remove the medium from the wells (Hek293 cells are easily dislodged from the plate). Make 3-fold serial dilutions of PMA in Assay Medium 1B. The final concentration of DMSO in assay medium in all dilutions should be 0.1%.

4. Add 100 µl of serial dilutions of PMA prepared in step 3 to stimulated wells.
   Add 100 µl of Assay Medium 1B with 0.1% DMSO to unstimulated control wells.
   Add 100 µl of Assay Medium 1B with 0.1% DMSO to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

5. Incubate the plate at 37°C in a CO2 incubator for ~ 6 hours.

6. 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. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

7. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The fold induction of AP1 luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

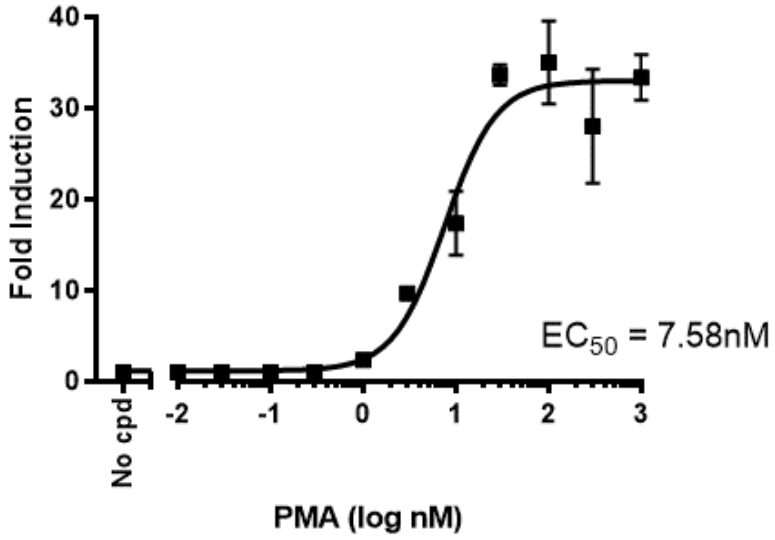

Figure 1. Dose response of AP1 Reporter – HEK293 cells to PMA.
The results are shown as fold induction of AP1 luciferase reporter expression.

B. Inhibition of PMA-induced reporter activity by an inhibitor of the JNK signaling pathway in AP1 Reporter – HEK293 cells

1. Harvest AP1 Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 1.

2. Incubate cells at 37°C in a CO2 incubator overnight.

3. The next day, carefully remove the medium from the wells (Hek293 cells are easily dislodged from the plate) and add 40 µl Assay Medium 1B. Dilute the inhibitor stock in Assay Medium 1B to 2x the final concentration desired.

4. Add 50 µl of diluted inhibitor in prepared in step 3 to the wells. The final concentration of DMSO in Assay Medium 1B can be up to 0.5%.

Add 50 µl of Assay Medium 1B with same concentration of DMSO without inhibitor to inhibitor control wells.
Add 100 µl of Assay Medium 1B with DMSO to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

5. Incubate the plate at 37°C in a CO2 incubator for 1 hour.

6. Add 10 µl of PMA diluted to 100nM in Assay Medium 1B to stimulated wells (final [PMA] = 10 nM).
Add 10 µl of Assay Medium 1B to the unstimulated control wells (cells without inhibitor and without PMA treatment for determining the basal activity).
Set up each treatment in at least triplicate.

Treatment Reference Guide

	Stimulated Wells		**Unstimulated Control Wells**	**Cell-free Control Wells**
	With Inhibitor	Without inhibitor (control well)		
Step 3 and 4	90 µl diluted inhibitor in Assay Medium 1B	90 µl Assay Medium 1B with DMSO only	90 µl Assay Medium 1B with DMSO only	100 µl Assay Medium 1B with DMSO only
Step 6	10 µl PMA in Assay Medium 1B (final [PMA] = 10 nM)	10 µl PMA in Assay Medium 1B (final [PMA] = 10 nM)	10 µl Assay Medium 1B with 0.1% DMSO	

7. Incubate the plate at 37°C in a 5% CO2 incubator for ~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. Measure luminescence using a luminometer.
If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

9. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The percent luminescence of AP1 luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells x 100%

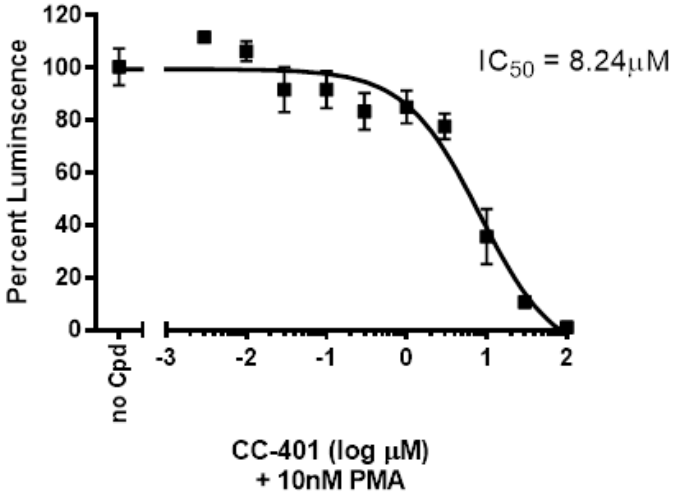

Figure 2. Inhibition of PMA-induced reporter activity by the JNK pathway inhibitor CC-401 in AP1 Reporter – HEK293 cell

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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*
Thaw Medium 1	60187	100 ml
Growth Medium 1B	79531	500 ml
AP1 Reporter Kit (JNK Signaling Pathway)	60612	500 reactions
SRE Reporter - HEK293 Cell line (ERK Pathway)	60406	2 vials
SRE Reporter Kit (MAPK/ERK Signaling Pathway)	60511	500 reactions
MAPK10 (JNK3), human	40092	10 µg
JNK1-β1(K55M), human	40871	100 µg
(NIK), human	40090	10 µg
MAPKAPK2 (MK2), human	40088	100 µg
JNK1, mouse	40071	10 µg
JNK2, human	40113	10 µg
ERK1, human	40055	10 µg
ERK2, human	40299	10 µg
ERK2, inactive, human	40056	10 µg
ONE-Step™ Luciferase Assay System	60690	10 ml/100 ml