6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

JNK Signaling Pathway
AP1 Reporter - HEK293 Recombinant Cell line
Catalog #: 60405

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. cJun 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 activators or inhibitors of the JNK signaling pathway.

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

Storage
Immediately upon receipt, store in liquid nitrogen.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life technologies #26140-079), 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 Cat. #79531) Thaw Medium 1 (BPS Cat. #60187) and 400 µg/ml of Geneticin (Life Technologies #11811031).

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1B (BPS Cat. #79531) (Thaw Medium 1 and Geneticin). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ 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 **(no Geneticin)**. 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)**. Cells should be split before they reach complete confluence.

To passage the cells, 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. Subcultivation ration: 1:10 to 1:20 weekly.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1B and transfer to a tube, spin down cells, and 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 in liquid nitrogen.

Functional Validation and Assay Performance
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.

Materials Required but Not Supplied for Cell Culture

- Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1B (BPS Cat. #79531)

Materials Required but Not Supplied for Cellular Assay

- Phorbol 12-Myristate 13-Acetate (PMA) (LC Laboratories # P-1680). Prepare stock solution in DMSO.
- JNK inhibitor V (AS601245) (Santa Cruz biotechnology # sc-202672): inhibitor of JNK1,2,3. Prepare stock solution in DMSO.
- Assay medium: Opti-MEM I (Life technologies # 31985-062), 0.5% FBS, 1% non-essential amino acids, 1mM Na pyruvate, and 1% Pen/Strep
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay Reagent (BPS Cat. #60690)
- Luminometer

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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.

A. Response of AP1 Reporter – HEK293 cells to PMA

1. Harvest AP1 Reporter – HEK293 cells from culture in Growth medium and seed cells at a density of ~ 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, carefully remove the medium from the wells. Make 3-fold serial dilutions of PMS in assay medium. The final concentration of DMSO in assay medium in all dilutions should be 0.1%.

4. Add 100 µl of serial dilutions of PMA in assay medium to stimulated wells.
   Add 100 µl of assay medium with 0.1% DMSO to unstimulated control wells.
   Add 100 µl of assay medium 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 CO_2 incubator for ~ 6 hours.

6. 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.
   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

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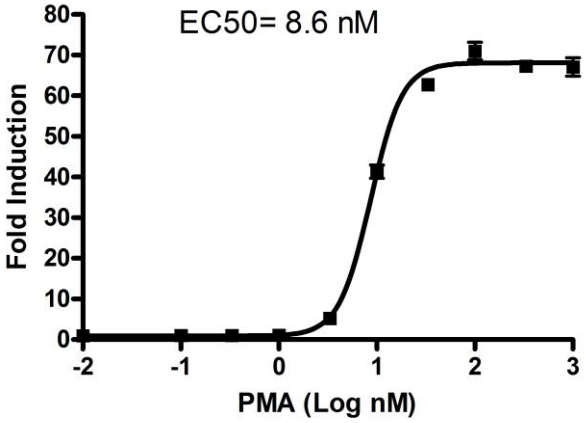
Figure 1. Dose response of AP1 Reporter – HEK293 cells to PMA.
The results are shown as fold induction of AP1 luciferase reporter expression.



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 and seed cells at a density of 35,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, dilute the inhibitor (JNK inhibitor V) stock in assay medium. Carefully remove the medium from wells and add 90 µl of diluted inhibitor in assay medium to the wells. The final concentration of DMSO in assay medium can be up to 0.5%.
   Add 90 µl of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
   Add 90 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).

4. Incubate the plate at 37°C in a CO_2 incubator for 1 hour.

5. Add 10 µl of diluted PMA in assay medium to stimulated wells (final [PMA] = 10 nM). Final DMSO concentration should be 0.1%.
   Add 10 µl of assay medium with 0.1% DMSO to the unstimulated control wells (cells without inhibitor and PMA treatment for determining the basal activity).

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Add 10 µl of assay medium with 0.1% DMSO to cell-free control wells.
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	90 µl diluted inhibitor in assay medium	90 µl assay medium with DMSO only	90 µl assay medium with DMSO only	90 µl assay medium with DMSO only
Step 5	10 µl PMA in assay medium (final [PMA] = 10 nM)	10 µl PMA in assay medium (final [PMA] = 10 nM)	10 µl assay medium with 0.1% DMSO	10 µl assay medium with 0.1% DMSO

6. Incubate the plate at 37°C in a CO_2 incubator for ~6 hours.

7. 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.
If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.

8. Data Analysis: Obtain background-subtracted luminescence by subtracting average background luminescence (cell-free control wells) from luminescence reading of all wells.

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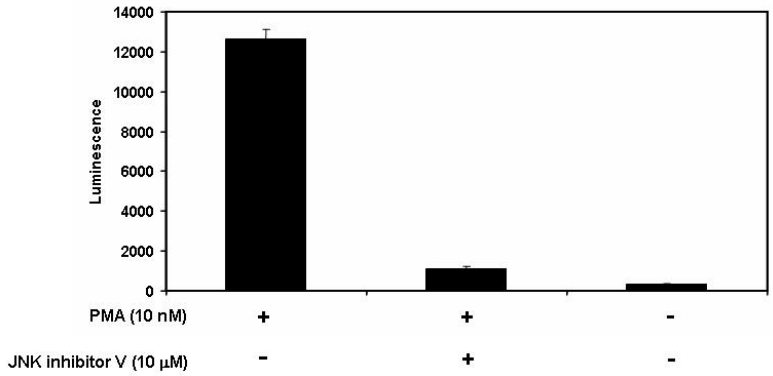
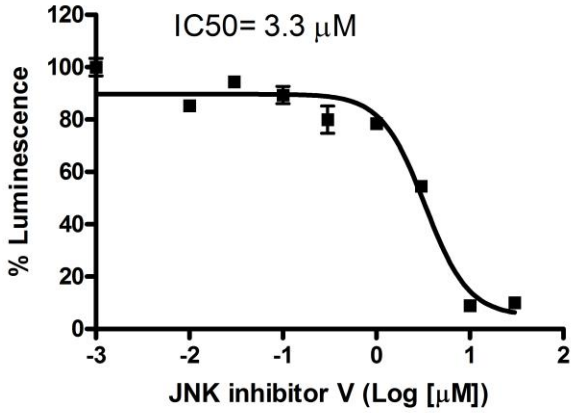
Figure 2. Inhibition of PMA-induced reporter activity by a JNK pathway inhibitor in AP1 Reporter – HEK293 cells

2a. JNK inhibitor V blocked PMA-induced AP1 reporter activity.



2b. JNK inhibitor V inhibition dose response curve

The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with PMA in the absence of JNK inhibitor V was set at 100%.



References:

1. Zhou H. *et. al*. (2005) Frequency and distribution of AP-1 sites in the human genome. *DNA Research.* **11:** 139-150.
2. Gaillard P. *et.al.* (2005) Design and synthesis of the first generation of novel potent, selective, and in vivo active (benzothiazol-2-yl)acetonitrile inhibitors of the c-Jun N-terminal kinase. *J Med Chem*. **48(14):**4596-4607.

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Related Products

Product Name:	**Catalog #**	**Size**
Thaw Medium 1	60187	100ml
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
MAP3K14 (NIK), human	40090	10 µg
MAPKAPK2 (MK2), human	40088	100 µg
JNK1, mouse	40071	10 µg
JNK2, human	40113	10 µg
JNK3, human	40114	10 µg
ERK1, human	40055	10 µg
ERK2, human	40299	10 µg
ERK2, inactive, human	40056	10 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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