Data Sheet

JNK Signaling Pathway
AP1 Reporter - HEK293 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.

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
- *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*
- *Luciferase reagents for measuring firefly luciferase activity (We use ONE-Glo™ luciferase assay system, Promega # E6110. Other luciferase assay systems are also suitable).*
- *Luminometer*

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 50 µl of serial dilutions of PMA in assay medium to stimulated wells.*
   Add 50 µl of assay medium with 0.1% DMSO to unstimulated control wells.
   Add 50 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.

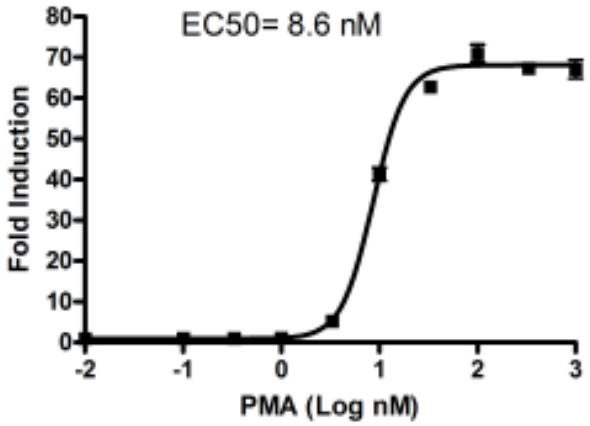
5. *Incubate the plate at 37°C in a CO_2 incubator for ~ 6 hours.*

6. *Perform luciferase assay using the ONE-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.*
   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.

The EC_{50} of PMA is ~ 8.6 nM



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 100µl 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 45 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 45 µl of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
   Add 45 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 5 µl of diluted PMA in assay medium to stimulated wells (final [PMA] = 10 nM). Final DMSO concentration should be 0.1%.*

Add 5 µl of assay medium with 0.1% DMSO to the unstimulated control wells (cells without inhibitor and PMA treatment for determining the basal activity).
Add 5 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	*45 µl diluted inhibitor in assay medium*	*45 µl assay medium with DMSO only*	*45 µl assay medium with DMSO only*	*45 µl assay medium with DMSO only*
Step 5	*5 µl PMA in assay medium (final [PMA] = 10 nM)*	*5 µl PMA in assay medium (final [PMA] = 10 nM))*	*5 µl assay medium with 0.1% DMSO*	*5 µl assay medium with 0.1% DMSO*

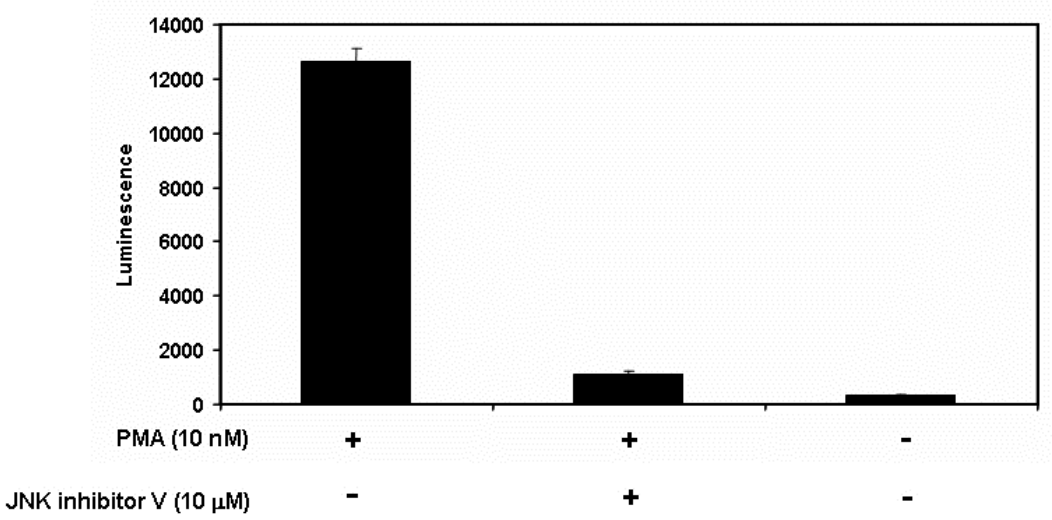
6. *Incubate the plate at 37°C in a CO_2 incubator for ~6 hours.*

7. *Perform luciferase assay using the ONE-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.*
   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.*

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

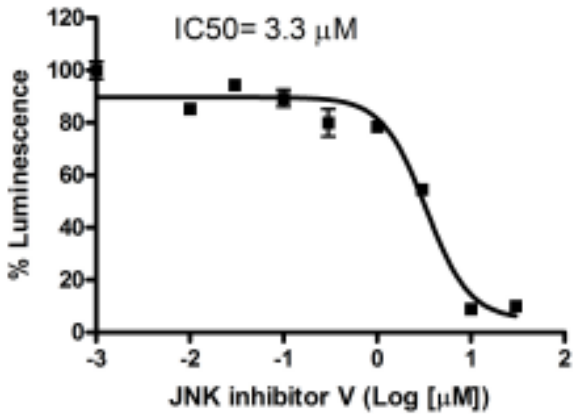
3a. JNK inhibitor V blocked PMA-induced AP1 reporter activity.



3b. 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%.

The IC_{50} of JNK inhibitor V is ~ 3.3 µM



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.

Culture conditions
Cells should be grown at 37°C with 5% CO_2 using 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), and 400 µg/ml of Geneticin (Life Technologies #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. 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), detach cells from culture vessel using 0.05% Trypsin/EDTA, add complete growth medium and 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.

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.*

Related Products

Product Name	*Catalog #*	*Size*
AP1 Reporter Kit (JNK Signaling Pathway)	*60612*	*500 reactions*
MAPK/ERK Signaling Pathway	*60406*	*1 vial*
SRE Reporter - HEK293 Cell line		
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*

License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales to your laboratory.

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