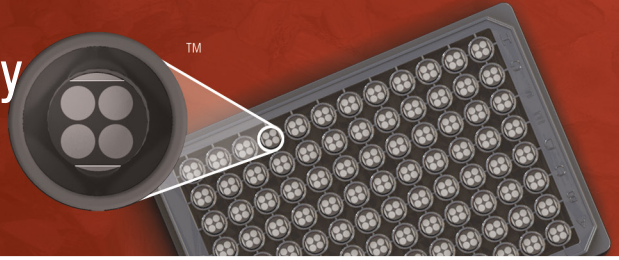
MSD® Phospho(Thr183/Tyr185)/Total JNK Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Phospho(Thr183/Tyr185)/Total JNK Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K15111D-1
5 plates	K15111D-2
20 plates	K15111D-3

Phospho-JNK Whole Cell Lysate Set	
200 µg	C11CU-1

Ordering information

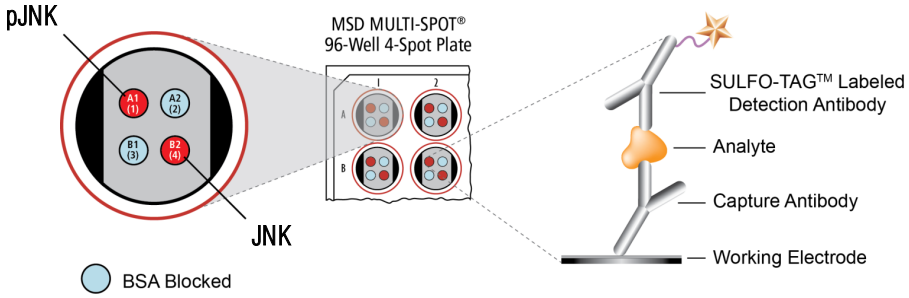
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA

www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures.



The **c-Jun N-terminal kinase (JNK)** family of kinases, also known as SAPK (stress-activated protein kinase) and MAPK8 (mitogen-activated protein kinase-8), are encoded by three genes (JNK 1-3) that produce more than 10 isoforms. Inflammatory cytokines and cellular stresses such as UV light and osmotic shock lead to JNK activation through the intracellular signaling cascade including Ras and Rac, MEKK1, and MKK4 or MKK7. JNK is fully activated by phosphorylation on threonine 183 and tyrosine 185 by MKK4 and MKK7. Activated JNK in turn phosphorylates the transcription factors c-Jun, ATF-2, and Elk-1, thereby regulating gene transcription. JNK is involved with the function of the mitochondrial apoptotic pathway, mediating the release of cytochrome c and the phosphorylation of Bim and Bmf. JNK also affects expression of many cytokine genes, as well as playing a role in tumor development, embryonic morphogenesis, and cell survival.

The MSD Phospho(Thr183/Tyr185)/Total JNK Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Thr183/Tyr185)/Total JNK Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-JNK (Thr183/Tyr185) and total JNK antibodies and are shown below for comparison.
Growing HEK293 cells were treated with rapamycin (1 µM; 3 hours) (negative). Serum deprived HEK293 cells were harvested 30 minutes after UV irradiation (40 mJ/cm^2) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4 Spot plates coated with anti-phospho-JNK (Thr183/Tyr185) antibody and anti-total JNK antibody on spatially distinct electrodes within a well. Phosphorylated and total JNK were detected with anti-total JNK antibody labeled with MSD SULFO-TAG™ reagent.

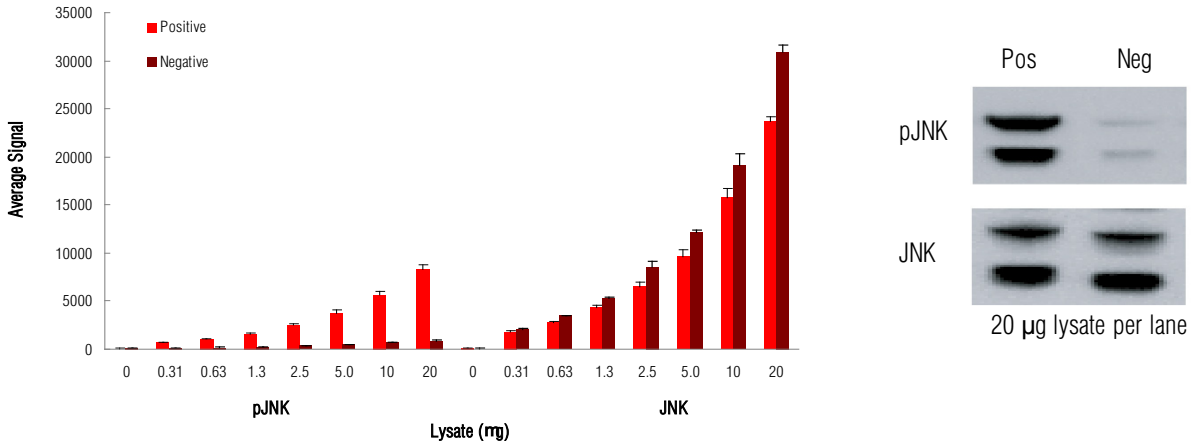


Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Thr183/Tyr185)/Total JNK Assay. Increased signal for phosphorylated JNK was observed with only pJNK positive cell lysate. Total JNK signal increased throughout the titration of both pJNK positive and negative cell lysates. The Phospho(Thr183/Tyr185)/Total JNK Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pJNK positive and negative HEK293 cell lysates using the MULTI-SPOT Phospho(Thr183/Tyr185)/Total JNK Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pJNK	0	57	13	23.4	61	12	18.9	
	0.31	704	31	4.4	128	22	17.1	5.5
	0.63	1031	43	4.1	170	26	15.5	6.1
	1.3	1576	140	8.9	259	10	3.9	6.1
	2.5	2517	174	6.9	362	11	2.9	6.9
	5.0	3759	381	10.1	465	5	1.0	8.1
	10	5598	408	7.3	672	34	5.0	8.3
	20	8337	494	5.9	873	39	4.4	9.5
JNK	0	66	4	5.8	56	9	15.3	
	0.31	1782	89	5.0	2069	51	2.5	0.9
	0.63	2764	92	3.3	3449	82	2.4	0.8
	1.3	4289	288	6.7	5290	110	2.1	0.8
	2.5	6484	479	7.4	8557	545	6.4	0.8
	5.0	9633	691	7.2	12128	283	2.3	0.8
	10	15727	1027	6.5	19175	1115	5.8	0.8
	20	23670	469	2.0	30898	757	2.5	0.8

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 µg/well or less without compromising speed or performance
- **Large dynamic range**: Linear range of up to five logs enables the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions
- **Minimal background:** The stimulation mechanism (electricity) is decoupled from the signal (light)
- **Simple protocols:** Only labels near the electrode surface are detected, enabling no-wash assays
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules
- **High sensitivity and precision:** Multiple excitation cycles of each label enhance light levels and improve sensitivity

For a complete list of products, please visit our website at www.mesoscale.com

References using MSD's platform for the measurement of phosphoproteins

1. Hua F, Henstock PV, Tang B. ERK activation by GM-CSF reduces effectiveness of p38 inhibitor on inhibiting TNFalpha release. Int Immunopharmacol. 2010 Jul;10(7):730-7.
2. Skepner JE, Shelley LD, Ji C, Reidich B, Luo Y. Chronic treatment with epoxyeicosatrienoic acids modulates insulin signaling and prevents insulin resistance in hepatocytes. Prostaglandins &Other Lipid Mediators. 2011 Feb;94(1-2):3-8.
3. Gowan SM, Hardcastle A, Hallsworth AE, Valenti MR, Hunter LJ, de Haven Brandon AK, Garrett MD, Raynaud F, Workman P, Aherne W, Eccles SA. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol. 2007 Jun;5(3):391-401.