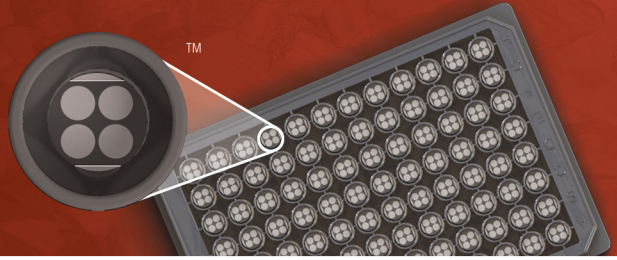
MSD® Total JNK Assay Whole Cell Lysate Kit

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

Total JNK Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150CVD-1
5 plates	K150CVD-2
20 plates	K150CVD-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®
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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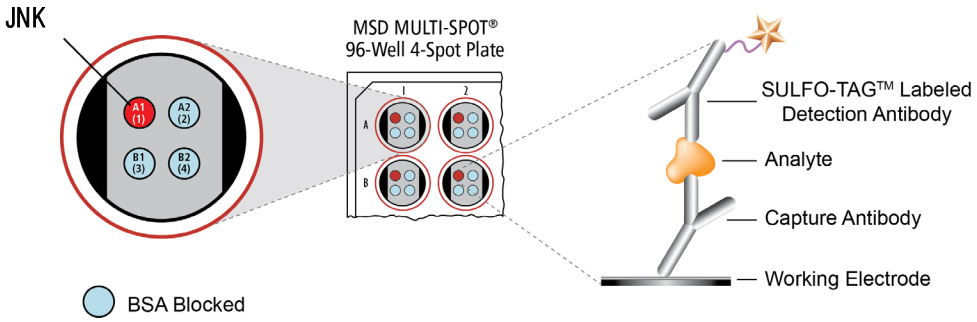

The **c-Jun N-terminal kinase (JNK)** family of kinases, also known as SAPK (stress-activated protein kinase) and MAPK8 (mitogen-activated protein kinase-8), are encoded by three genes (JNK 1-3) that produce more than 10 isoforms. Inflammatory cytokines and cellular stresses such as UV light and osmotic shock lead to JNK activation through the intercellular 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 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 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-total JNK antibody on one of the four spatially distinct electrodes per well. Total JNK was detected with anti-total JNK antibody labeled with MSD SULFO-TAG™ reagent.

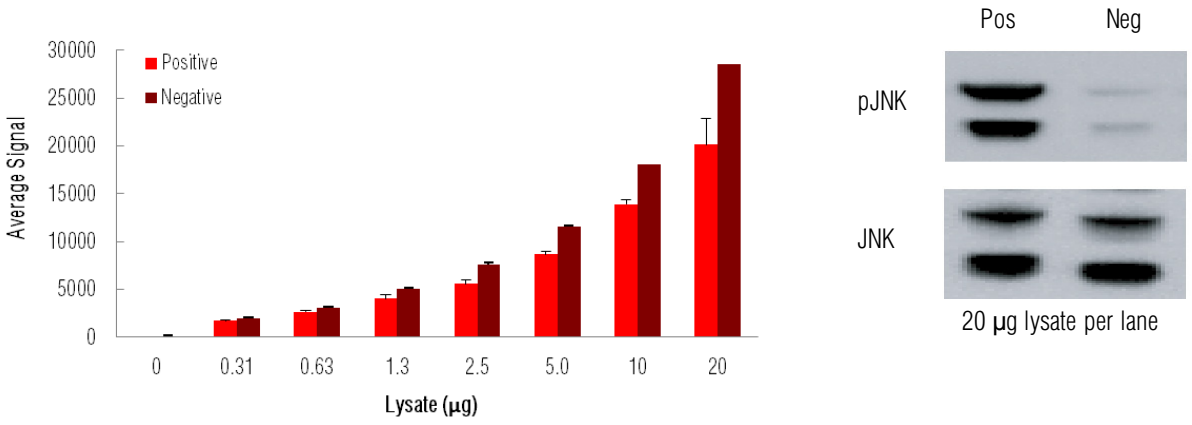

Fig. 1: Sample data generated with the MULTI-ARRAY® Total JNK Assay. Increased signal is observed with the titration of both pJNK positive and negative cell lysates. The Total JNK Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pJNK positive and negative HEK293 cell lysates using the MULTI-ARRAY Total JNK Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	60	13	22.1	80	14	17.1	
0.31	1724	130	7.5	1981	76	3.8	0.9
0.63	2676	108	4.1	3034	332	10.9	0.9
1.3	4040	377	9.3	5019	551	11.0	0.8
2.5	5602	343	6.1	7644	462	6.0	0.7
5.0	8689	302	3.5	11523	640	5.6	0.8
10	13883	455	3.3	18111	1252	6.9	0.8
20	20117	2700	13.4	28539	1864	6.5	0.7

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

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References using MSD's platform for the measurement of phosphoproteins

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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.
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4. 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.