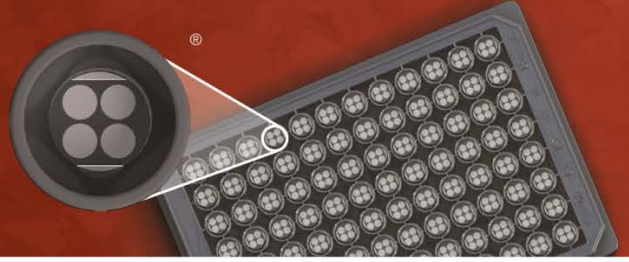
MSD® MAP Kinase (Total Protein) Assay: Whole Cell Lysate Kit

For quantitative determination of p38, ERK1/2, and JNK in human, mouse, and rat whole cell lysate samples



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

Catalog Numbers

MAP Kinase (Total Protein) Assay: Whole Cell Lysate Kit	
Kit Size	**Catalog #**
1 plate	K15157D-1
5 plates	K15157D-2
20 plates	K15157D-3

Phospho-MAPK Whole Cell Lysate Set	
200 µg	C1101-1

Ordering Information

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

Scientific Support

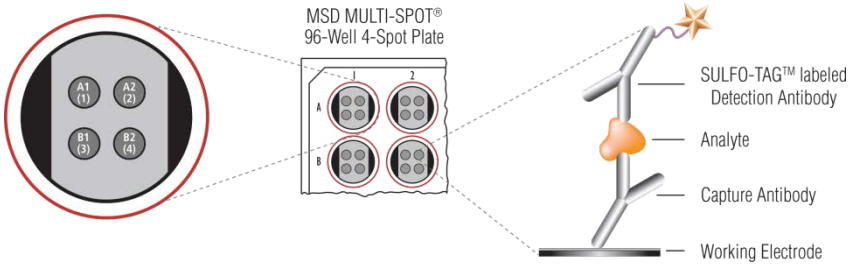
Phone: 1-301-947-2025
Email: ScientificSupport@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com®

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

1. p38
2. *BSA blocked*
3. JNK
4. ERK1/2



MAP (Mitogen-Activated Protein) kinases are a family of evolutionarily conserved eukaryotic serine/threonine protein kinases which link receptors on the cell surface to important intracellular regulatory targets. MAP kinases also elicit an intracellular effect in response to physical and chemical cellular stress. MAP kinase cascades within the cell are composed of a series of proteins, the first of which is a MAP kinase kinase kinase (MAPKKK).[1] The MAPKKK is activated by phosphorylation in response to growth factors, mitogens, inflammatory cytokines, G-protein coupled receptors (GPCRs) or stress. The MAPKKK in turn phosphorylates MAPKK, which then phosphorylates MAPK.[2] The activated terminal MAPK translocates into the nucleus, thereby exerting an effect on gene transcription. Through these pathways, the cell regulates responses leading to cell proliferation and differentiation, development, inflammation, and cell survival or apoptosis.[2] ERK1/2, p38, and JNK are all MAP kinases, activated by the MAPK kinases MEK1/2, MKK3/6, and MKK4/7, respectively.[2] Because these pathways are critical for the regulation of cell growth and survival, the MAP kinase family of enzymes offers desirable targets for the development of anti-cancer therapeutics.[3]

The MSD MAP Kinase (Total Protein) Assay Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the MAP Kinase (Total Protein) Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested.
Growing Jurkat cells were treated with rapamycin (1 mM; 3 hours) (negative) or with PMA (200 nM) and calyculin A (50 nM) for 30 minutes (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total p38, anti-total JNK, and anti-total ERK1/2 antibodies on spatially distinct electrodes per well. Total p38, JNK, and ERK1/2 were detected with anti-total p38, anti-total JNK, and anti-total ERK1/2 antibodies conjugated with MSD SULFO-TAG™ reagent.

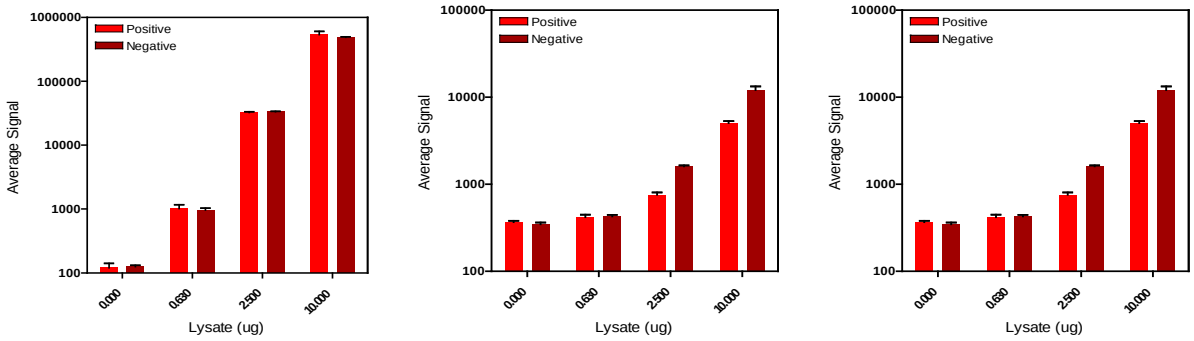


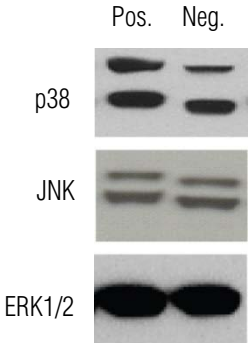
Figure 1: Sample data generated with MULTI-SPOT MAP Kinase (Total Protein) Assay. Increased signals were observed with the titration of both phospho-MAPK positive and negative cell lysates.

Lysate Titration

Data for positive and negative Jurkat cell lysates using the MULTI-SPOT MAP Kinase (Total Protein) Assay are presented below.

	Lysate (µg)/well	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
p38	0	121	21	17.0	126	6	5.1	
	0.63	1026	151	14.7	967	66	6.8	1.1
	2.5	32 549	1028	3.2	33 565	371	1.1	1.0
	10	538 146	64 754	12.0	488 214	9487	1.9	1.1
ERK1/2	0	143	1	0.5	162	12	7.4	
	0.63	225	23	10.1	343	9	2.7	0.7
	2.5	1002	52	5.2	2340	28	1.2	0.4
	10	7038	295	4.2	18 537	1554	8.4	0.4
JNK	0	367	13	3.7	346	16	4.7	
	0.63	419	29	6.9	428	17	4.0	1.0
	2.5	758	49	6.4	1602	41	2.6	0.5
	10	5033	287	5.7	12 110	1136	9.4	0.4

Western blot analyses of each lysate type were performed with p38, ERK1/2, and JNK antibodies and are shown below for comparison. The MAP Kinase (Total Protein) Assay provides a measure of the data obtained with the traditional Western blot.



20 µg lysate per lane

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

References

1. Keshet Y, Seger R. The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. Mol Cancer Ther. Methods Mol Biol. 2010;661:3-38.
2. Zhang YL, Dong C. MAP kinases in immune responses. Cell Mol Immunol. 2005 Feb;2(1):20-7.
3. Dhillon AS, et al. MAP kinase signalling pathways in cancer. Oncogene. 2007 May 14;26(22):3279-90.