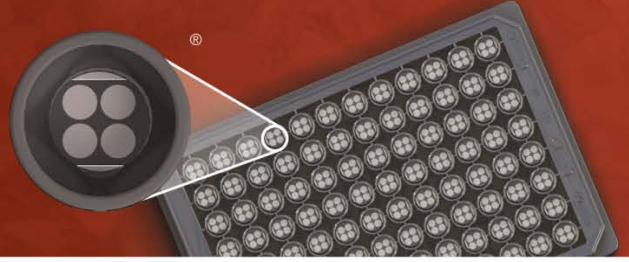
MSD® MAP Kinase Phosphoprotein Assay: Whole Cell Lysate Kit

For quantitative determination of phosphorylated p38(Thr180/Tyr182), ERK1/2 (Thr202/Tyr204; Thr185/Tyr187), and JNK (Thr183/Tyr185) 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 Phosphoprotein Assay: Whole Cell Lysate Kit	
Kit Size	**Catalog #**
1 plate	K151OD-1
5 plates	K151OD-2
20 plates	K151OD-3

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

Ordering Information

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

Scientific Support

Phone: 1-301-947-2025
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Company Address

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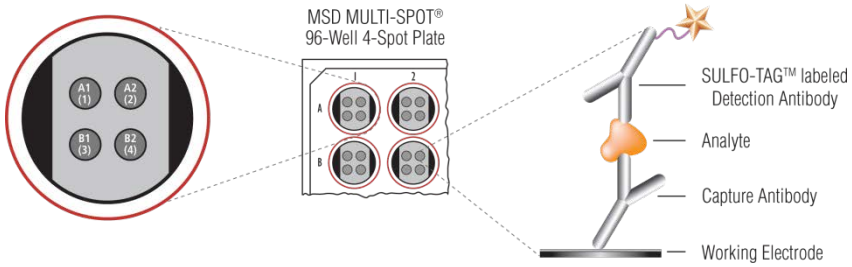


MAP (Mitogen-Activated Protein) kinases are a family of evolutionarily conserved eukaryotic serine/threonine protein kinases which link receptors on the cell surface to important intracellular regulatory targets. MAP kinases also elicit an intracellular effect in response to physical and chemical cellular 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 Phosphoprotein 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 Phosphoprotein 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 µM; 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-phospho-p38, anti-total JNK, and anti-phospho-ERK1/2 antibodies on spatially distinct electrodes per well. Phosphorylated p38, JNK, and ERK1/2 were detected with anti-total p38, anti-phospho-JNK, and anti-total ERK1/2 antibodies conjugated with MSD SULFO-TAG™ reagent.

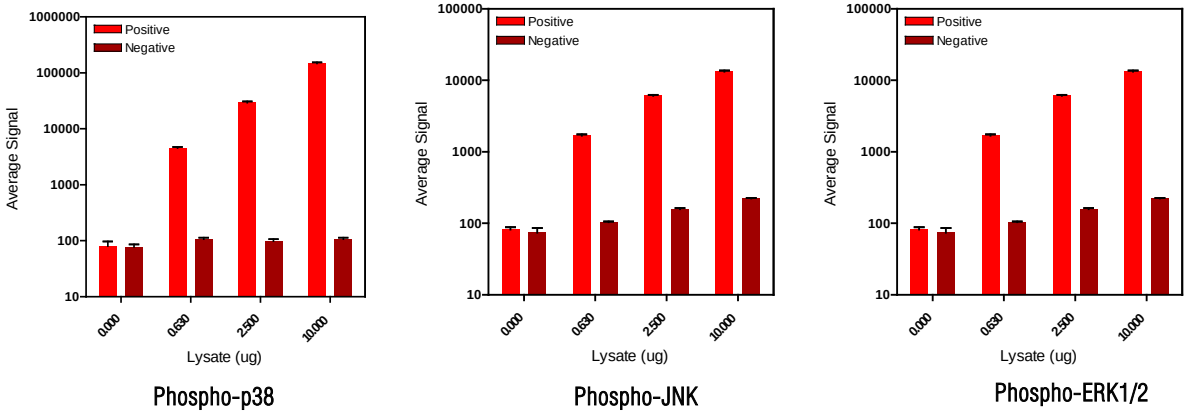


Figure 1: Sample data generated with MULTI-SPOT MAP Kinase Phosphoprotein Assay. Increased signals for phosphorylated forms of p38, pERK1/2, and pJNK were observed with only Phospho-MAPK positive cell lysate. Signals for negative lysate remained low throughout the titration.

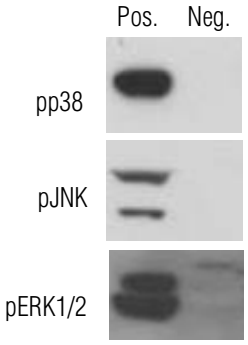
Spot the Difference®

Lysate Titration

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

	Lysate (µg)/well	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
Phospho-p38	0	80	17	21	77	9	11.5	
	0.625	4529	190	4.2	107	6	5.5	42
	2.5	29363	1498	5.1	99	7	7.2	297
	10	147217	6772	4.6	106	7	6.8	1389
Phospho-ERK1/2	0	82	6	7.6	74	9	11.8	
	0.625	1691	61	3.6	104	2	2.1	16
	2.5	6095	165	2.7	158	9	5.9	39
	10	13253	477	3.6	223	7	3	59
Phospho-JNK	0	138	17	12.3	127	4	3.4	
	0.625	4398	97	2.2	178	9	4.9	25
	2.5	13145	342	2.6	232	6	2.8	57
	10	24542	1939	7.9	305	12	3.8	80

Western blot analyses of each lysate type were performed with phospho-p38, phospho-ERK1/2, and phospho-JNK antibodies and are shown below for comparison. The MAP Kinase Phosphoprotein 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.