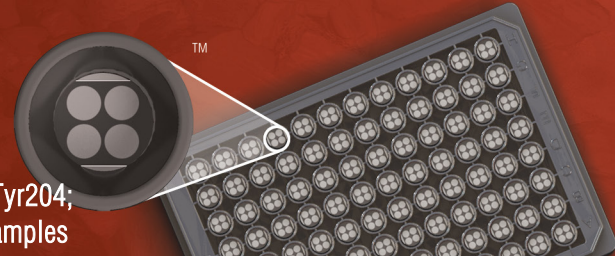


MSD® MAP Kinase Phosphoprotein Assay: Whole Cell Lysate Kit

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
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

MAP Kinase Phosphoprotein Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K15101D-1
5 plates	K15101D-2
20 plates	K15101D-3

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

Ordering information

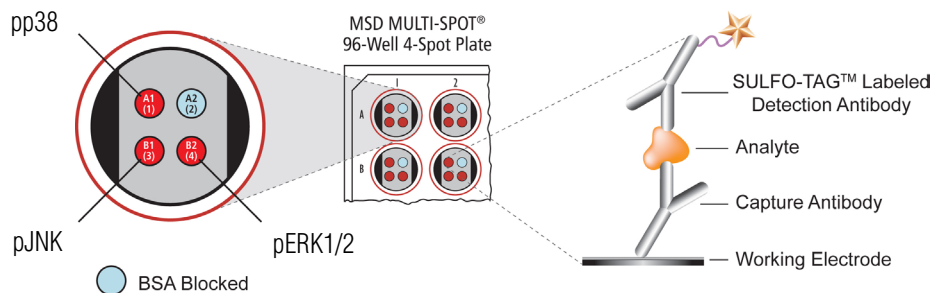
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

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). 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. 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. ERK1/2, p38, and JNK are all MAP kinases, activated by the MAPK kinases MEK1/2, MKK3/6, and MKK4/7, respectively. 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.

The MSD MAP Kinase Phosphoprotein 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. 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.

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.

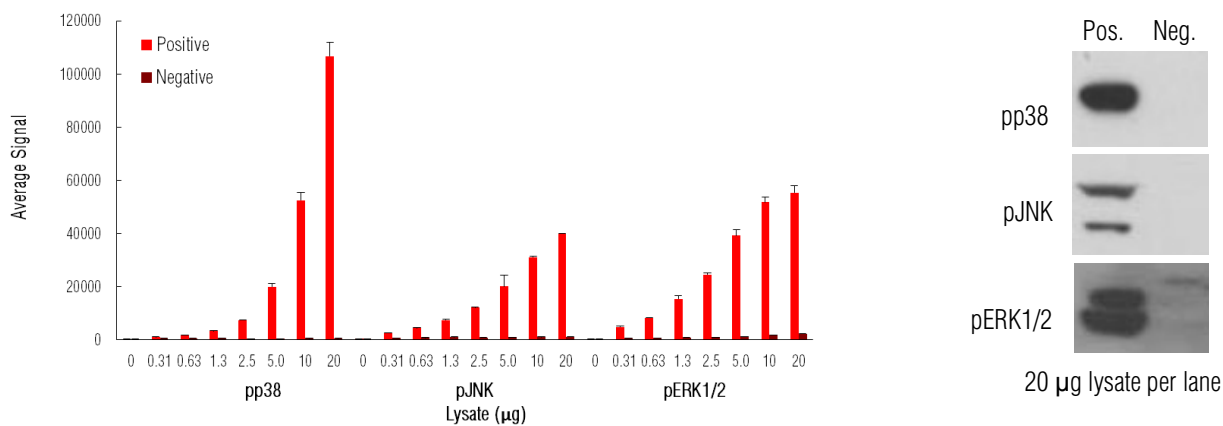


Fig. 1: Sample data generated with MULTI-SPOT MAP Kinase Phosphoprotein Assay. Increased signals for phosphorylated forms of p38, pERK1/2, and pJNK were observed with only Phospho-MAPK positive cell lysate. Signals for negative lysate remained low throughout the titration. The MAP Kinase Phosphoprotein Assay provides a quantitative measure of the data obtained with the traditional Western blot.

MSD Phosphoprotein Assays

Lysate Titration

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

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pp38	0	315	3	0.9	267	34	12.7	
	0.31	1,029	48	4.7	517	33	6.4	2.0
	0.63	1680	48	2.9	567	31	5.5	3.0
	1.3	3310	116	3.5	610	1	0.1	5.4
	2.5	7479	35	0.5	466	23	5.0	16
	5.0	20019	1044	5.2	472	34	7.2	42
	10	52545	2834	5.4	490	18	3.8	107
	20	106686	5220	4.9	487	32	6.5	219
pJNK	0	413	16	3.8	432	18	4.3	
	0.31	2618	170	6.5	704	0	0.0	3.7
	0.63	4449	225	5.1	775	17	2.2	5.7
	1.3	7404	317	4.3	1012	127	12.6	7.3
	2.5	12085	346	2.9	888	36	4.1	14
	5.0	20075	4387	6.9	963	37	3.9	21
	10	31085	499	1.6	1063	29	2.7	29
	20	40008	151	0.4	1142	25	2.2	35
pERK1/2	0	247	7	2.9	238	1	0.6	
	0.31	4860	282	5.8	533	2	0.4	9.1
	0.63	8219	252	3.1	664	8	1.2	12
	1.3	15380	1172	7.6	850	2	0.2	18
	2.5	24486	666	2.7	956	18	1.9	26
	5.0	39234	2245	5.7	1257	28	2.3	31
	10	51812	1895	3.7	1804	25	1.4	29
	20	55368	2645	4.8	2297	33	1.4	24

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

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