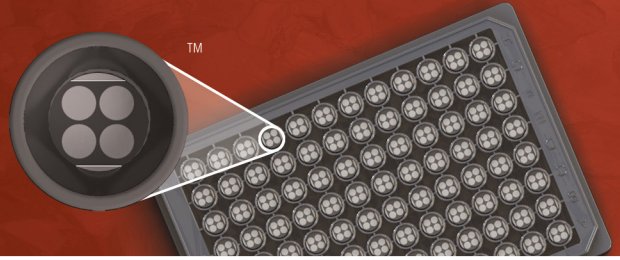
MSD® Phospho-MEK1/2 (Ser217/221) Assay Whole Cell Lysate Kit

For quantitative determination 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

Phospho-MEK1/2 (Ser217/221) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151CWD-1
5 plates	K151CWD-2
20 plates	K151CWD-3

Phospho-MEK1/2 Whole Cell Lysate Set	
200 µg	C11CW-1

Ordering information

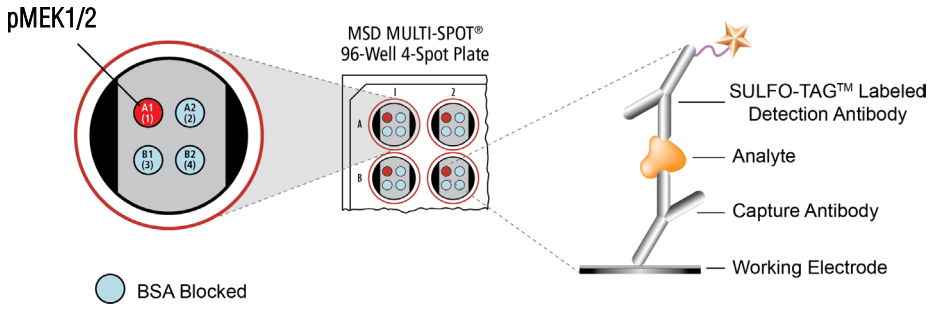
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

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.

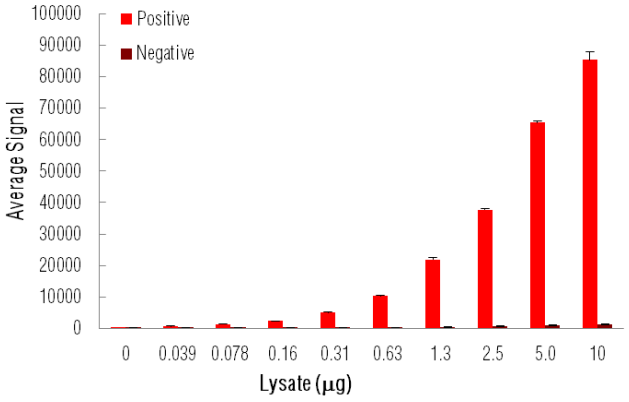


MEK1 and **MEK2** (MAPK/ERK kinases 1 and 2), also known as MKK1 and MKK2, are dual-specificity kinases that function as part of the intracellular mitogen-activated protein kinase signaling cascade activated in response to cellular stimulation by cytokines and growth factors. MEK1 and MEK2 are phosphorylated by the serine/threonine kinases Raf-1, Mos, and MEK kinase on serines 217 and 221. PDK1 has also been shown to phosphorylate MEK1 and MEK2, linking the PI-3 kinase /Akt signaling pathway with ERK activation. Activated MEK1 and MEK2 phosphorylate ERK1 /2 on threonine 202 and tyrosine 204 of ERK1 and threonine 185 and tyrosine 187 of ERK2. Activated ERK1/2 phosphorylate targets in both the nucleus and cytoplasm, exerting a regulatory effect on both transcription and translation. The activation of the Raf/MEK/ERK pathway has been shown to affect development, cell growth and differentiation, cell transformation, and cell cycle progression.

The MSD Phospho-MEK1/2 (Ser217/221) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-MEK1/2 (Ser217/221) 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-MEK1/2 (Ser217/221) and total MEK1/2 antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells were treated with PMA (200 nM; 15 minutes) (positive) or LY294002 (50 µM; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total MEK1/2 antibodies on one of the four spatially distinct electrodes within a well. Phosphorylated MEK1/2 was detected with anti-phospho-MEK1/2 (Ser217/221) antibody conjugated with MSD SULFO-TAG™ reagent.



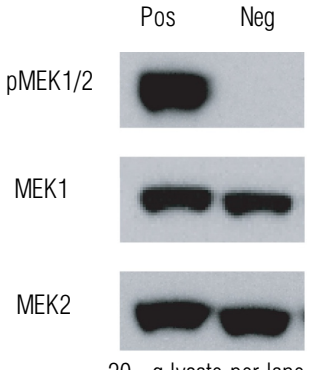


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-MEK1/2 (Ser217/221) Assay. Increased signal is observed with the titration of pMEK1/2 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-MEK1/2 (Ser217/221) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pMEK1/2 positive and negative Jurkat cell lysates using the MULTI-ARRAY Phospho-MEK1/2 (Ser217/221) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	81	9	11.0	81	9	11.0	
0.039	708	43	6.1	62	9	15.0	12
0.078	1321	48	3.6	94	7	7.0	14
0.16	2360	71	3.0	122	4	3.0	19
0.31	5047	228	4.5	181	15	8.0	28
0.63	10288	331	3.2	221	7	3.0	47
1.3	21894	575	2.6	375	12	3.0	58
2.5	37639	509	1.4	616	24	4.0	61
5.0	65371	661	1.0	884	9	1.0	74
10	85377	2624	3.1	1179	57	5.0	72

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. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koeppen H, Belvin M, Friedman LS, Malek S. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010 Mar 18;464(7287):431-5. Epub 2010 Feb 3.
2. Jaulmes A, Sansilvestri-Morel P, Rolland-Valognes G, Bernhardt F, Gaertner R, Lockhart BP, Cordi A, Wierzbicki M, Rupin A, Verbeuren TJ. Nox4 mediates the expression of plasminogen activator inhibitor-1 via p38 MAPK pathway in cultured human endothelial cells. Thromb Res. 2009 Sep;124(4):439-46. Epub 2009 Jun 21.
3. Martin SE, Jones TL, Thomas CL, Lorenzi PL, Nguyen DA, Runfola T, Gunsior M, Weinstein JN, Goldsmith PK, Lader E, Huppi K, Caplen NJ. Multiplexing siRNAs to compress RNAi-based screen size in human cells. Nucleic Acids Res. 2007;35(8):e57. Epub 2007 Mar 28.
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.