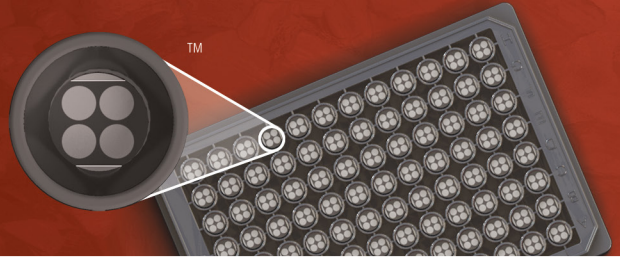
MSD® Phospho-ERK5 (Thr218/Tyr220) Assay Whole Cell Lysate Kit

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

Phospho-ERK5 (Thr218/Tyr220) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151IVD-1
5 plates	K151IVD-2
20 plates	K151IVD-3

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
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Company Address

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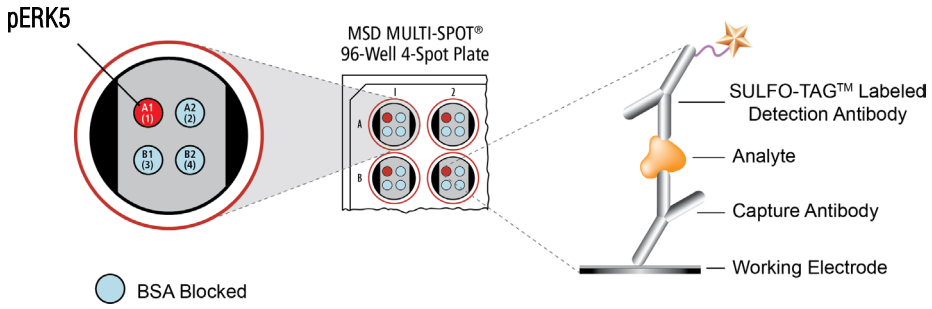

ERK 5 (Extracellular signal related kinase 5), also known as big mitogen activated protein kinase 1 (BMPK1) and mitogen activated protein kinase 7 (MAPK7) is approximately 123 kDa and is an atypical member of the MAP kinase superfamily.[1] ERK5 is activated via phosphorylation on its signature Thr-Glu-Tyr motif by MEK5 and plays a role in such cellular processes as proliferation, differentiation, transcriptional regulation, and development.[2] ERK 5 has many similarities to ERK1/2 in terms of activation stimuli as well as downstream signaling partners, but there are also some notable differences between these two MAPKs.
In vivo, ERK5 plays a role in cardiovascular development and neuronal differentiation.[2] Multiple MAPKs are also transcription factors and ERK5 is no exception. ERK5, unlike other MAPK family members, has a large C-terminal region which is involved in transcriptional regulation.[1,3] ERK 5 is normally located in both the cytoplasm and the nucleus, depending upon the cell line, but an increased concentration is seen in the nucleus upon stimulation through the MEK5 pathway.[4] The potential role ERK5 plays in cancer and heart disease make it an interesting target for drug development to modulate this signaling cascade.[5]

The MSD Phospho-ERK5 (Thr218/Tyr220) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-ERK5 (Thr218/Tyr220) 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-ERK5 (Thr218/Tyr220) and total ERK5 antibodies and are shown below for comparison.
Confluent HeLa cell monolayers (negative) were treated with sorbitol (0.4 M, 30 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total ERK5 antibody on one of the four spatially distinct electrodes within a well. Phosphorylated ERK5 was detected with anti-phospho-ERK5 antibody conjugated with MSD SULFO-TAG™ reagent.

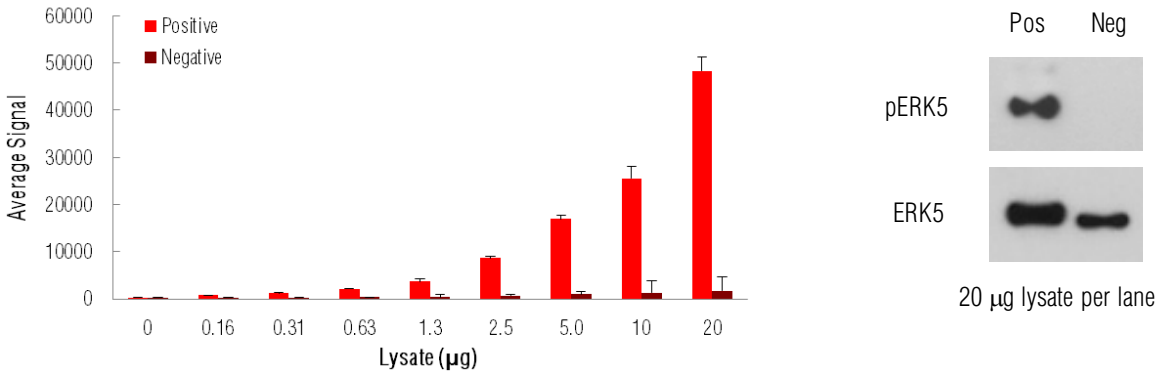

Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-ERK5 (Thr218/Tyr220) Assay. Increased signal is observed with the titration of pERK5 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-ERK5 (Thr218/Tyr220) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pERK5 positive and negative HeLa cell lysates using the MULTI-ARRAY Phospho-ERK5 (Thr218/Tyr220) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	316	34	10.8	316	34	10.8	
0.16	795	12	1.5	323	8	2.4	2.5
0.31	1264	44	3.5	345	8	2.3	3.7
0.63	2138	44	2.1	415	33	7.8	5.2
1.3	3747	607	16.2	424	52	12.2	8.8
2.5	8670	302	3.5	735	47	6.3	12
5.0	17018	674	4.0	984	78	7.9	17
10	25606	2544	9.9	1297	21	1.6	20
20	48359	2939	6.1	1732	35	2.0	28

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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3. Akaike M, Che W, Marmarosh NL, Ohta S, Osawa M, Ding B, Berk BC, Yan C, Abe J. The hinge-helix 1 region of peroxisome proliferator-activated receptor gamma1 (PPARgamma1) mediates interaction with extracellular signal-regulated kinase 5 and PPARgamma1 transcriptional activation: involvement in flow-induced PPARgamma activation in endothelial cells. Mol Cell Biol. 2004 Oct;24(19):8691-704.
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