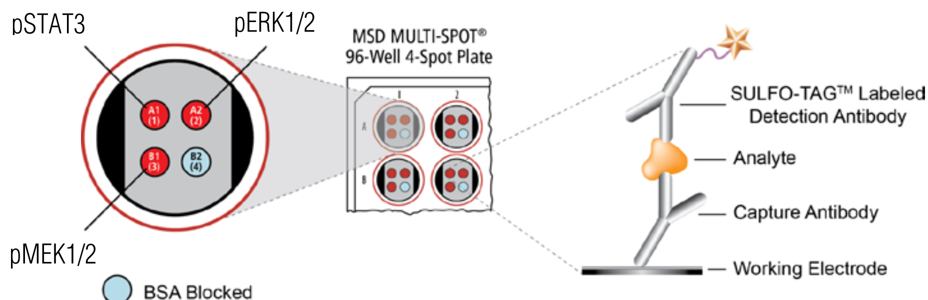
MSD® ERK-STAT3 Cascade Assay Whole Cell Lysate Kit

For quantitative determination of phospho-STAT3 (Tyr705), phospho-ERK1/2 (Thr202/Tyr204; Thr185/Tyr187), and phospho-MEK1/2 (Ser217/221) in human, mouse, and rat whole cell lysate

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



The Extracellular Signal-Regulated Kinase 1/2 (ERK1/2) belongs to the mitogen-activated protein (MAP) kinases family of eukaryotic, serine/threonine protein kinases which link cell surface receptors to important intracellular regulatory targets. The ERK1/2 cascade is a central signaling pathway that regulates more than 100 different substrates and regulates a wide variety of cellular processes including proliferation, differentiation, cell survival, and stress responses.[1] MAP and ERKs are activated by diverse mechanisms including ligation of receptor tyrosine kinases such as epidermal growth factor (EGF) and cell adhesion receptors such as the integrins. Ligand binding of these receptors leads to activation of the small GTPase Ras, which recruits Raf to the membrane, where it is activated. Raf subsequently phosphorylates the dual specificity **MAP/ERK kinase (MEK1/2)** at Ser217 and Ser221 and this activated kinase then phosphorylates ERK at both Thr202 and Tyr204 residues.[1,2] This event results in full activation of ERK1/2 and leads to its binding of several scaffold or adaptor proteins, such as **Signal Transducer and Activator of Transcription 3 (STAT3)**. This leads to various subcellular and nuclear localization and transactivation of critical regulatory genes.[1,3]

The MSD ERK-STAT3 Cascade Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Catalog Numbers

ERK-STAT3 Cascade Assay Whole Cell Lysate Kit	
Kit size	
1 plate	K15116D-1
5 plates	K15116D-2
20 plates	K15116D-3

Ordering information

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Typical Data with HeLa Cells Treated with Oncostatin M:

Representative results for the ERK-STAT3 Cascade Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Confluent HeLa cells (negative) were treated with sodium vanadate (1 mM, 4 hours) and oncostatin M (40 ng/mL, 5 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-ERK1/2, anti-total MEK1/2, and anti-total STAT3 antibodies on three of the four spatially distinct electrodes per well. Phosphorylated ERK1/2, MEK1/2 and STAT3 were detected with anti-total ERK1/2, anti-phospho-MEK1/2, and anti-phospho-STAT3 antibodies conjugated with MSD SULFO-TAG™ reagent. Western blot analyses of each lysate type were performed with phospho-ERK1/2, phospho-MEK1/2, and phospho-STAT3 antibodies and are shown below for comparison.

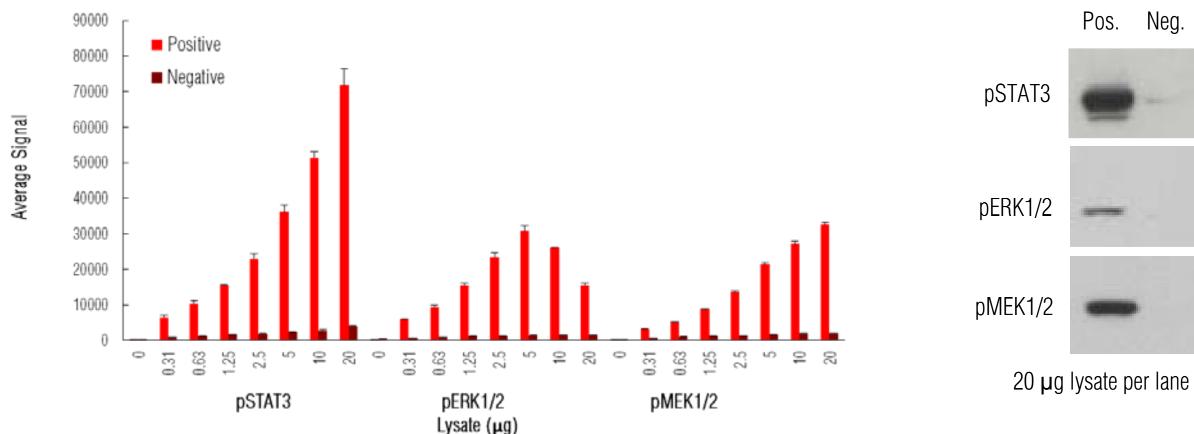

Fig. 1: Sample data generated with MULTI-SPOT ERK-STAT3 Cascade Assay. Increased signals were observed with only ERK-STAT3 Cascade positive cell lysate. Signals for negative lysate remained low throughout the titration for all assays. The ERK-STAT3 Cascade Assay provides a quantitative measure of the data obtained with the traditional Western blot.

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Lysate Titration

Data for positive and negative HeLa cell lysates using the MULTI-SPOT ERK-STAT3 Cascade Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pSTAT3	0	282	4	1.5	284	6	2.0	
	0.31	6460	557	8.6	937	11	1.1	6.9
	0.63	10314	776	7.5	1262	38	3.0	8.2
	1.3	15509	186	1.2	1664	14	0.8	9.3
	2.5	22944	1607	7.0	1885	18	0.9	12
	5.0	36039	2024	5.6	2279	114	5.0	16
	10	51344	1749	3.4	2824	271	9.6	18
	20	71772	4660	6.5	3941	88	2.2	18
pERK1/2	0	346	26	7.6	382	30	8.0	
	0.31	5847	243	4.2	720	26	3.6	8.1
	0.63	9469	449	4.7	923	76	8.3	10
	1.3	15579	570	3.7	1232	25	2.0	13
	2.5	23598	1155	4.9	1320	18	1.3	18
	5.0	30672	1792	5.8	1500	75	5.0	20
	10	26111	29	0.1	1589	16	1.0	16
	20	15584	537	3.4	1507	15	1.0	10
pMEK1/2	0	324	7	2.2	338	2	0.6	
	0.31	3181	89	2.8	778	18	2.4	4.1
	0.63	5215	20	0.4	1026	38	3.7	5.1
	1.3	8896	8	0.1	1330	13	1.0	6.7
	2.5	13667	318	2.3	1419	18	1.2	9.6
	5.0	21299	492	2.3	1696	1	0.0	13
	10	27110	759	2.8	1978	22	1.1	14
	20	32608	550	1.7	1989	34	1.7	16

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Typical Data with Jurkat Cells Treated with PMA: Control Lysates for Phosphorylated ERK1/2 and MEK1/2

Representative results for the ERK-STAT3 Cascade Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Jurkat cells were treated with LY294002 (50 µM, 2.5 hours) (negative) or with PMA (200 nM, 15 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-ERK1/2, anti-total MEK1/2, and anti-total STAT3 antibodies on three of the four spatially distinct electrodes per well. Phosphorylated ERK1/2, MEK1/2, and STAT3 were detected with anti-total ERK1/2, anti-phospho-MEK1/2, and anti-phospho-STAT3 antibodies conjugated with MSD SULFO-TAG reagent. Western blot analyses of each lysate type were performed with phospho-ERK1/2, phospho-MEK1/2, and phospho-STAT3 antibodies and are shown below for comparison.

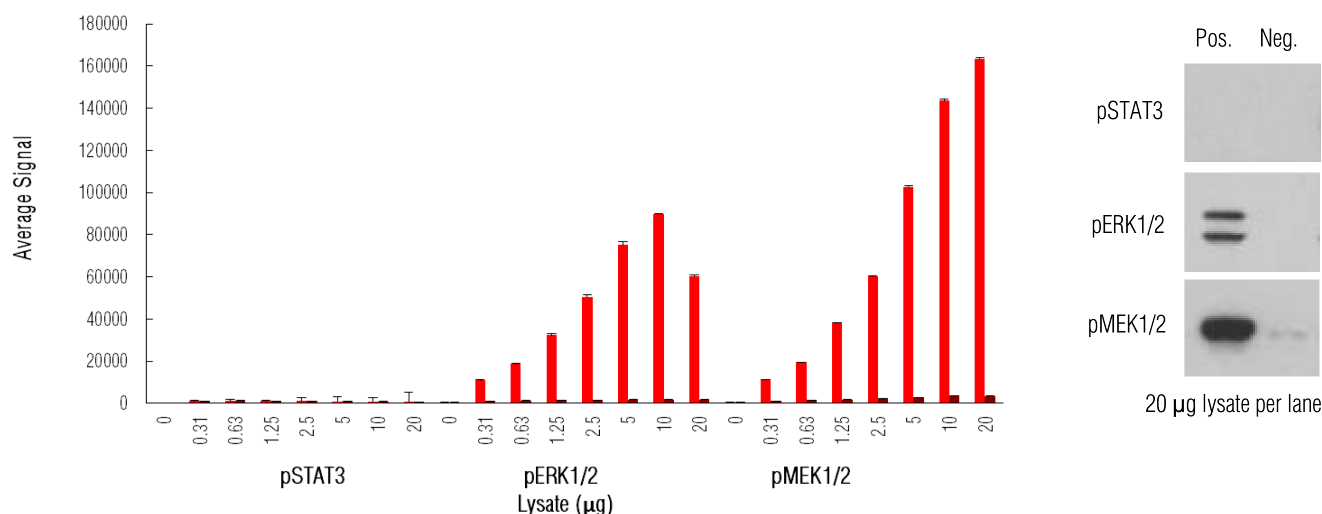

Fig. 2: Sample data generated with MULTI-SPOT ERK-STAT3 Cascade Assay. Increased signals were observed for pERK1/2 and pMEK1/2 assays with the titration of ERK-STAT3 Cascade positive cell lysate. Signals for pSTAT3 assay remained low. Signals for negative lysate also remained low throughout the titration for all assays. The ERK-STAT3 Cascade Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative Jurkat cell lysates using the MULTI-SPOT ERK-STAT3 Cascade Assay are presented below.

	Lysate	Positive			Negative			P/N
	(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pSTAT3	0	310	11	3.6	314	22	7.0	
	0.31	999	46	4.6	975	0	0.0	1.0
	0.63	1170	62	5.3	1209	23	1.9	1.0
	1.3	1146	10	0.9	1134	23	2.0	1.0
	2.5	1000	13	1.3	974	62	6.3	1.0
	5.0	897	40	4.4	851	15	1.7	1.1
	10	798	25	3.1	755	13	1.7	1.1
	20	735	0	0.0	642	4	0.7	1.1

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	Lysate (µg)	Positive Average Signal	Positive StdDev	Positive %CV	Negative Average Signal	Negative StdDev	Negative %CV	P/N
pERK1/2	0	365	20	5.4	389	17	4.4	
	0.31	10840	472	4.4	904	0	0.0	12
	0.63	18559	854	4.6	1196	38	3.2	16
	1.3	32432	2232	6.9	1481	136	9.2	22
	2.5	50489	2063	4.1	1579	105	6.7	32
	5.0	75075	2758	3.7	1752	100	5.7	43
	10	89922	4707	5.2	1746	9	0.5	52
	20	60208	2896	4.8	1650	35	2.1	36
pMEK1/2	0	346	29	8.4	349	13	3.9	
	0.31	11108	352	3.2	1102	41	3.7	10
	0.63	19518	817	4.2	1523	49	3.2	13
	1.3	38011	575	1.5	1812	66	3.6	21
	2.5	60152	928	1.5	2031	47	2.3	30
	5.0	102748	4020	3.9	2819	21	0.7	36
	10	143528	1480	1.0	3388	8	0.3	42
	20	163450	364	0.2	3454	17	0.5	47

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:

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2. Frémin C, Meloche S. From basic research to clinical development of MEK1/2 inhibitors for cancer therapy. J Hematol Oncol. 2010 Feb 11;3:8.
3. Lim CP, Cao X. Structure, function, and regulation of STAT proteins. Mol Biosyst. 2006 Nov;2(11):536-50.

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