MSD[®] Phospho-STAT3 (Tyr705) 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-STAT3 (Tyr705) Assay: Whole Cell Lysate Kit						
Kit size						
1 plate	K150DID-1					
5 plates	K150DID-2					
20 plates	K150DID-3					

Phospho-STAT3 (Tyr705) Whole Cell Lysate Set					
200 μ g	C11DI-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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STAT3 is one of a family of cytoplasmic transcription factors activated by cytokines, growth factors, and hormones. Phosphorylation of STAT3 on tyrosine 705 results in its activation and subsequent dimerization, nuclear translocation, and DNA binding. In response to cellular stimulation by cytokines, STAT3 phosphorylation is mediated through the JAK family of receptor associated tyrosine kinases, most notably JAK1. Growth factor receptors with intrinsic tyrosine kinase activities may phosphorylate STAT3 directly, and the non-receptor tyorsine kinase SRC has been shown to phophorylate STAT3 as well. Activated STAT3 has been shown to play a critical role in cellular processes including proliferation, tissue-dependent cell survival of apoptosis, and embryonic development and organogenesis. Constitutively activated STAT3 has been observed in breast, skin, prostate, lung, and breast cancers.

The MSD Phospho-STAT3 (Tyr705) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-STAT3 (Tyr705) 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-STAT3 (Tyr705) and total STAT3 antibodies and are shown below for comparison.

Confluent Hela cells (negative) were pretreated with sodium vanadate (1 mM; 4 hours) and stimulated with Oncostatin M (40 ng/mL; 5 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT[®] 4-Spot plates coated with anti-total STAT3 antibody on one of the four spatially distinct electrodes per well. Phosphorylated STAT3 was detected with anti-phospho-STAT3 (Tyr705) antibody conjugated with MSD SULFO-TAG[™] reagent.



Fig. 1: Sample data generated with the MULTI-ARRAY[®] Phospho-STAT3 (Tyr705) Assay. Increased signal is observed with the titration of pSTAT3 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-STAT3 (Tyr705) Assay provides a quantitative measure of the data obtained with the traditional Western blot.





Lysate Titration

Data for pSTAT3 positive and negative HeLa cell lysates using the MULTI-ARRAY Phospho-STAT3 (Tyr705) Assay are presented below.

Lysate	Positive			Negative			D/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	F/N
0	120	9	7.2	120	9	7.2	
0.019	269	10	3.7	116	5	4.6	2.3
0.039	428	20	4.7	134	27	20.0	3.2
0.078	709	23	3.2	141	4	3.0	5.0
0.16	1224	58	4.7	169	9	5.2	7.2
0.31	1978	61	3.1	216	6	2.9	9.2
0.63	3582	116	3.2	300	2	0.7	12
1.3	6294	241	3.8	459	4	0.9	14
2.5	11105	166	1.5	971	28	2.8	11
5.0	19524	376	1.9	1265	23	1.8	15
10	36095	1204	3.3	2335	71	3.1	15
20	64751	1119	1.7	4287	127	3.0	15

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

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- Lam LT, Wright G, Davis RE, Lenz G, Farinha P, Dang L, Chan JW, Rosenwald A, Gascoyne RD, Staudt LM. Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-{kappa}B pathways in subtypes of diffuse large B-cell lymphoma. od. 2008 Apr 1;111(7):3701-13. Epub 2007 Dec 26.
- 3. 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.

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