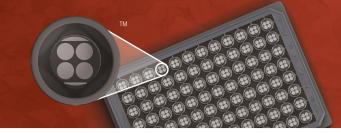
# MSD® Total STAT5a,b Assay Whole Cell Lysate Kit

For quantitative determination in human and mouse whole cell lysate samples



Alzheimer's Disease BioProcess Cardiac

#### Cell Signaling

Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

#### Catalog Numbers

Total STAT5a,b Assay: Whole Cell Lysate Kit				
Kit size				
1 plate	K150IHD-1			
5 plates	K150IHD-2			
20 plates	K150IHD-3			

	Phospho-STAT5a,b (Tyr694) Whole Cell Lysate Set					
200 μ <b>g</b>	C10IG-1					

#### Ordering information

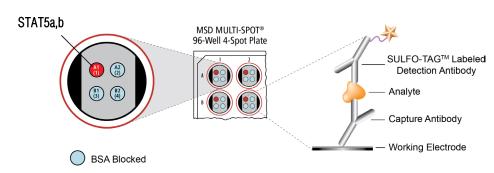
MSD Customer Service Phone: 1-301-947-2085 Fax: 1-301-990-2776 Email: CustomerService@ mesoscale.com

#### Company Address

MESO SCALE DISCOVERY® A division of Meso Scale Diagnostics, LLC. 9238 Gaither Road Gaithersburg, MD 20877 USA

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**Signal Transducer and Activator of Transcription 5a and 5b (STAT 5a and 5b)** are members of the STAT family of transcription factors, part of the Jak/STAT signal transduction pathway, and are approximately 90% identical at the amino acid level. When inactive, STATs are cytoplasmic; upon ligand binding and activation of a cytokine receptor, the receptor binds to a member of the Jak family. The receptor is phosphorylated, recruits a member of the STAT family, which is also phosphorylated, dimerizes, and is then transported into the nucleus where it can act as a transcription factor.<sup>1</sup>

STAT5a,b are involved in many different types of cancer (such as breast and prostate), inflammatory responses, and allergic reactions.<sup>2</sup> STAT5s also play a role in preventing apoptosis in certain cell types and diseases, based upon their role as transcription factors and the genes they regulate.<sup>3</sup> Due to the important transcriptional control of the STAT family of proteins, their role in intracellular signaling of many different cytokines, and their importance in diseases such as cancer and inflammation, there has been much research and pharmacological study of this very important signaling pathway.

The MSD Total STAT5a,b Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

#### Typical Data

Representative results for the Total STAT5a,b 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 total STAT5a and total STAT5b antibodies and are shown below for comparison.

Confluent HeLa cells (negative) were pretreated with Na-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 STAT5a and anti-total STAT5b antibodies on one of the four spatially distinct electrodes per well. Total STAT5a,b was detected with an anti-total STAT5 antibody conjugated with MSD SULFO-TAG™ reagent.

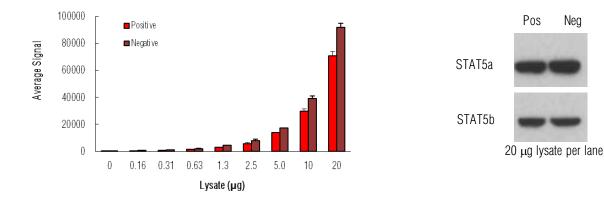


Fig. 1: Sample data generated with the MULTI-ARRAY® Total STAT5a,b Assay. Increased signal is observed with the titration of both pSTAT5a,b positive and negative cell lysates. The Total STAT5a,b Assay provides a quantitative measure of the data obtained with the traditional Western blot.





## MSD Phosphoprotein Assays

#### Lysate Titration

Data for pSTAT5a,b positive and negative HeLa cell lysates using the MULTI-ARRAY Total STAT5a,b Assay are presented below.

Lysate	Positive			Negative			D/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	P/N
0	112	9	7.9	112	9	7.9	
0.16	466	15	3.1	660	27	4.1	0.7
0.31	750	175	23.4	1105	58	5.3	0.7
0.63	1615	71	4.4	2125	159	7.5	0.8
1.3	3023	264	8.7	4408	32	0.7	0.7
2.5	5545	1171	21.1	8068	273	3.4	0.7
5.0	13806	202	1.5	17305	1312	7.6	0.8
10	29773	1577	5.3	39281	2078	5.3	0.8
20	70832	3034	4.3	91723	846	0.9	0.8

### 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

- 1. Clevenger CV. Roles and Regulation of Stat Family Transcription Factors in Human Breast Cancer. Am J Pathol. 2004 Nov;165(5):1449-60.
- 2. Morales JK, Falanga YT, Depcrynski A, Fernando J, Ryan JJ. Mast cell homeostasis and the JAK–STAT pathway. Genes Immun. 2010 Dec;11(8):599-608. Epub 2010 Jun 10.
- 3. Mekori YA, Gilfillan AM, Akin C, Hartmann K, Metcalfe DD. Human mast cell apoptosis is regulated through Bcl-2 and Bcl-XL. J Clin Immunol. 2001 May;21(3):171-4.

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