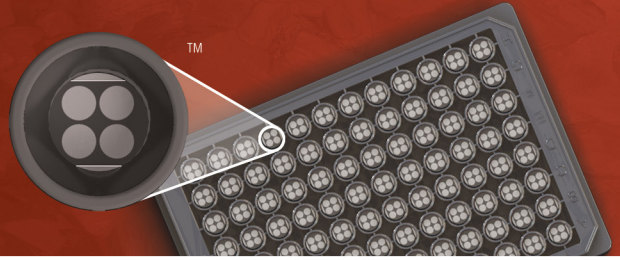
# MSD® Phospho(Tyr694)/Total STAT5a,b Assay Whole Cell Lysate Kit

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

| Phospho(Tyr694)/Total STAT5a,b: Whole Cell Lysate Kit | |
|---|---|
| Kit size | |
| 1 plate | K15163D-1 |
| 5 plates | K15163D-2 |
| 20 plates | K15163D-3 |

| Phospho-STAT5a,b (Tyr694) Whole Cell Lysate Set | |
|---|---|
| 200 µg | C10IG-1 |

## Ordering information

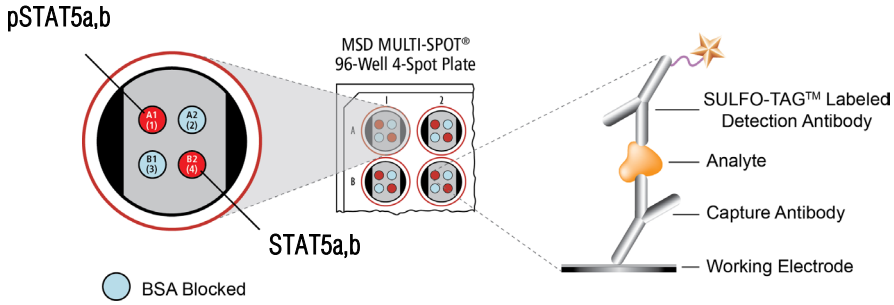
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

## Company Address

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

**www.mesoscale.com®**

**For Research Use Only. Not for use in diagnostic procedures.**




**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.[1]
STAT5a,b are involved in many different types of cancer (such as breast and prostate), inflammatory responses, and allergic reactions.[2] 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.[3] 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 Phospho(Tyr694)/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 Phospho(Tyr694)/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 phospho-STAT5a,b (Tyr694) and total STAT5a,b 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-phospho-STAT5a,b (Tyr694) and anti-total STAT5a,b antibodies on spatially distinct electrodes within a well. Phosphorylated and total STAT5a,b were detected with anti-total STAT5a,b antibody with MSD SULFO-TAG™ reagent.

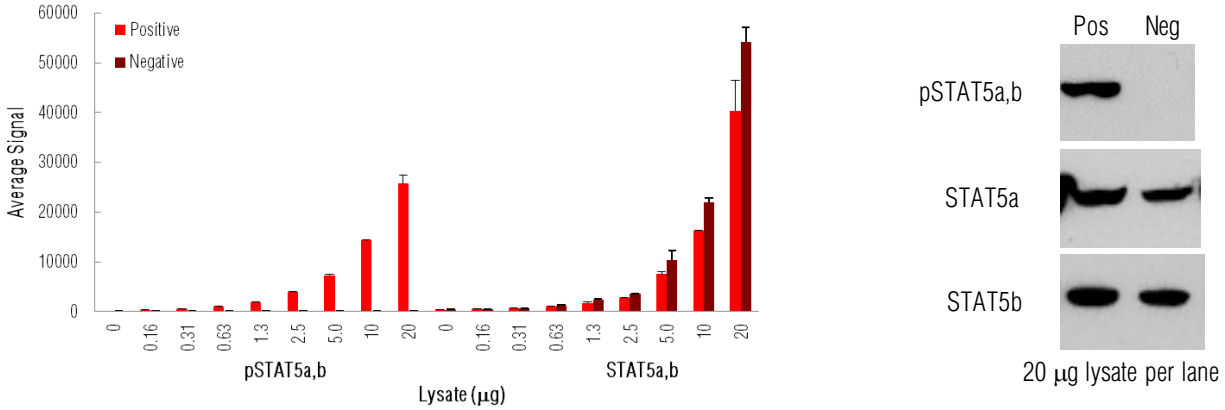



**Fig. 1:** Sample data generated with the MULTI-SPOT® Phospho(Tyr694)/Total STAT5a,b Assay. Increased signal for phosphorylated STAT5a,b was observed with only pSTAT5a,b positive cell lysate. Total STAT5a,b signal increased throughout the titration of both pSTAT5a,b positive and negative cell lysates. The Phospho(Tyr694)/Total STAT5a,b Assay provides a quantitative measure of the data obtained with the traditional Western blot.

## Lysate Titration

Data for pSTAT5a,b positive and negative HeLa cell lysates using the MULTI-SPOT Phospho(Tyr694)/Total STAT5a,b Assay are presented below.

| | Lysate (µg) | Positive | | | Negative | | | P/N |
|---|---|---|---|---|---|---|---|---|
| | | Average Signal | StdDev | %CV | Average Signal | StdDev | %CV | |
| pSTAT5a,b | 0 | 89 | 7 | 7.7 | 89 | 7 | 7.7 | |
| | 0.16 | 288 | 16 | 5.7 | 66 | 3 | 4.3 | 4.4 |
| | 0.31 | 529 | 42 | 7.9 | 73 | 0 | 0.0 | 7.2 |
| | 0.63 | 959 | 61 | 6.3 | 71 | 19 | 27.1 | 14 |
| | 1.3 | 1867 | 44 | 2.3 | 87 | 8 | 9.0 | 22 |
| | 2.5 | 4021 | 35 | 0.9 | 68 | 6 | 9.4 | 64 |
| | 5.0 | 7287 | 141 | 1.9 | 114 | 4 | 3.7 | 64 |
| | 10 | 14256 | 196 | 1.4 | 132 | 1 | 1.1 | 108 |
| | 20 | 25753 | 1615 | 6.3 | 171 | 14 | 8.3 | 151 |
| STAT5a,b | 0 | 349 | 46 | 13.2 | 349 | 46 | 13.2 | |
| | 0.16 | 457 | 32 | 7.0 | 476 | 36 | 7.6 | 1.0 |
| | 0.31 | 660 | 6 | 1.0 | 681 | 0 | 0.0 | 1.0 |
| | 0.63 | 998 | 5 | 0.5 | 1244 | 89 | 7.2 | 0.8 |
| | 1.3 | 1712 | 148 | 8.6 | 2407 | 99 | 4.1 | 0.7 |
| | 2.5 | 2769 | 82 | 3.0 | 3522 | 14 | 0.4 | 0.8 |
| | 5.0 | 7545 | 419 | 5.6 | 10287 | 1925 | 18.7 | 0.7 |
| | 10 | 16159 | 120 | 0.7 | 22007 | 775 | 3.5 | 0.7 |
| | 20 | 40319 | 6171 | 15.3 | 54094 | 3017 | 5.6 | 0.7 |

## 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.


MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, WWW.MESOSCALE.COM, MSD, MSD (DESIGN), DISCOVERY WORKBENCH, QUICKPLEX, MULTI-ARRAY, MULTI-SPOT, SULFO-TAG, SECTOR, SECTOR HTS, SECTOR PR, 4-SPOT (DESIGN) and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC.
© 2011 Meso Scale Diagnostics, LLC. All rights reserved.

**For Research Use Only. Not for use in diagnostic procedures.**