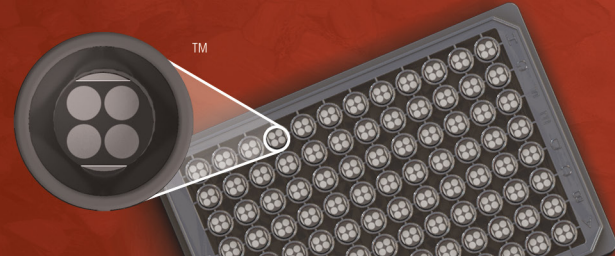


# MSD® Phospho-STAT5a,b (Tyr694) Assay Whole Cell Lysate Kit

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-STAT5a,b (Tyr694) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150IGD-1
5 plates	K150IGD-2
20 plates	K150IGD-3

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

## Ordering information

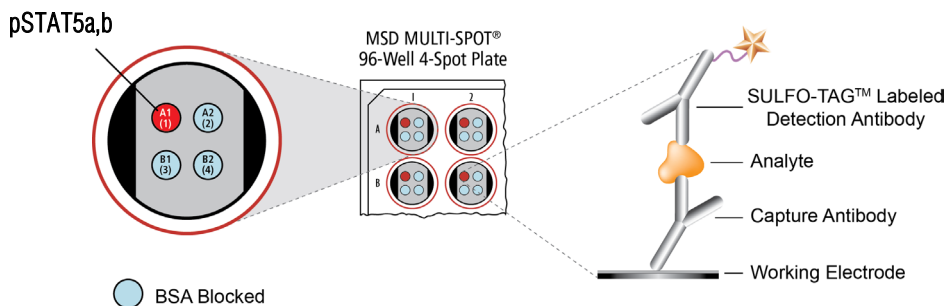
MSD Customer Service  
Phone: 1-301-947-2085  
Fax: 1-301-990-2776  
Email: CustomerService@mesoscale.com

## Company Address

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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.<sup>1</sup>

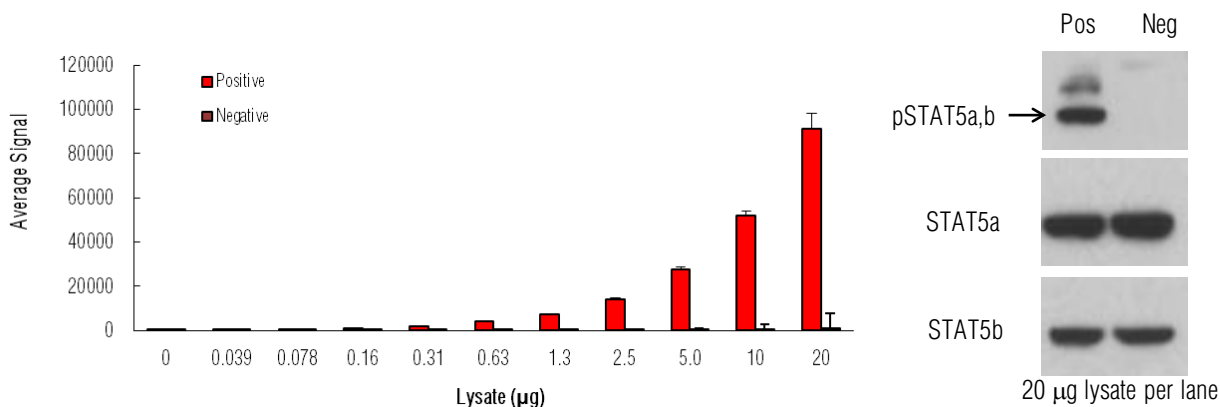
STAT5a,b are involved in many different types of cancer (such as breast and prostate), inflammatory responses, and allergic reactions.<sup>2</sup> STAT5s also play a role in preventing apoptosis in certain cell types and diseases, based upon their role as transcription factors and the genes they regulate.<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 Phospho-STAT5a,b (Tyr694) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

## Typical Data

Representative results for the Phospho-STAT5a,b (Tyr694) 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 antibody on one of the four spatially distinct electrodes per well. Phosphorylated STAT5a,b was detected with an anti-total STAT5a,b antibodies conjugated with MSD SULFO-TAG™ reagent.



**Fig. 1:** Sample data generated with the MULTI-ARRAY® Phospho-STAT5a,b (Tyr694) Assay. Increased signal is observed with the titration of pSTAT5a,b positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-STAT5a,b (Tyr694) 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 Phospho-STAT5a,b (Tyr694) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	118	22	18.2	79	9	10.8	
0.039	373	14	3.7	75	4	5.1	5.0
0.078	660	20	3.0	92	5	5.4	7.2
0.16	1167	26	2.2	110	6	5.0	11
0.31	2069	119	5.8	90	6	6.1	23
0.63	3921	72	1.8	111	8	6.8	35
1.3	7419	129	1.7	160	12	7.2	46
2.5	14242	278	1.9	211	12	5.6	67
5.0	27796	813	2.9	314	3	0.9	89
10	51964	2079	4.0	505	30	6.0	103
20	91331	6684	7.3	905	54	5.9	101

## 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](http://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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