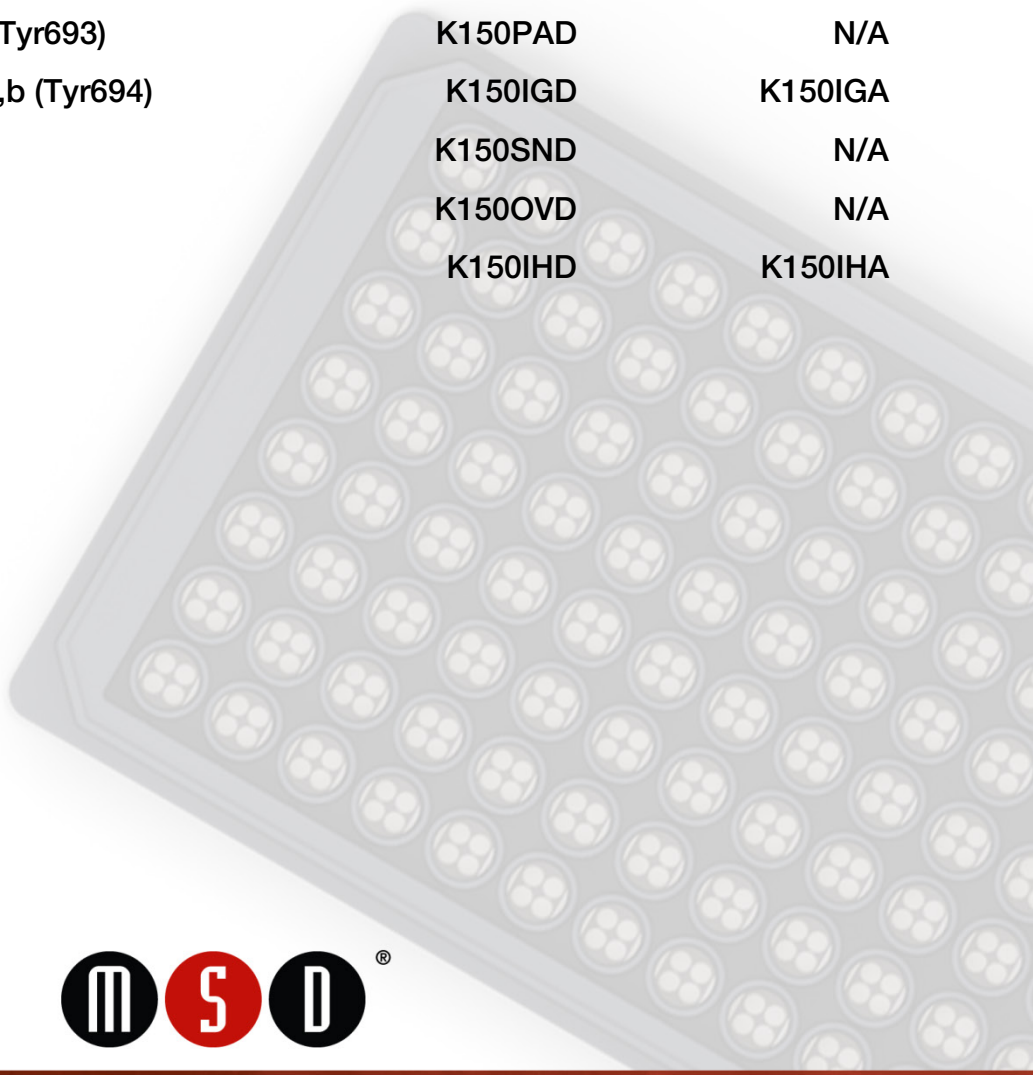
MSD® MULTI-SPOT Assay System

STAT Kits

	Complete Kit	Base Kit
Phospho-STAT Panel	K15202D	N/A
Singleplex Kits		
Phospho-STAT3 (Tyr705)	K150SVD	N/A
Phospho-STAT4 (Tyr693)	K150PAD	N/A
Phospho-STAT5a,b (Tyr694)	K150IGD	K150IGA
Total STAT3	K150SND	N/A
Total STAT4	K150OVD	N/A
Total STAT5a,b	K150IHD	K150IHA



MSD Cell Signaling Pathway Assays

STAT Kits

For use with human, mouse, and rat cell lysates.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
A division of Meso Scale Diagnostics, LLC.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

Table of Contents

Contact Information

MSD Customer Service

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

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Signal Transducer and Activator of Transcription 3 (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 tyrosine kinase SRC phosphorylates STAT3 as well. Activated STAT3 plays 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 skin, prostate, lung, and breast cancers.

STAT4 is a 748 amino acid member of the STAT family expressed in myeloid cells, thymus, and testis. STAT4 has multiple functional domains, including an N-terminal interaction domain, a central DNA-binding domain, an SH2 domain, and the C-terminal transactivation domain. Activation of STAT4 is initiated by IL-12–mediated signaling. IL-12, a cytokine secreted by antigen-presenting cells, binds to transmembrane receptors, resulting in receptor heterodimerization and activation of the Janus kinases JAK2 and TYK2.[1,2] These tyrosine kinases activate STAT4 by phosphorylation of tyrosine 693; active STAT4 homodimerizes through the SH2 domain and translocates into the nucleus to activate gene transcription of cytokines as well as cytokine receptors and other proteins.[3] Haplotypes for STAT4 are associated with increased risk for rheumatoid arthritis and systemic lupus erythematosus.[4,5]

STAT 5a and 5b are members of the STAT family of transcription factors, part of the JAK/STAT signal transduction pathway, and 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 and recruits a member of the STAT family that is then phosphorylated. It then dimerizes and is transported into the nucleus, where it can act as a transcription factor.[6] In all, there are about 38 cytokines that signal through their cytokine receptors and through the JAK/STAT pathway to cause downstream transcriptional effects. Interestingly, these effects seem to be cell-type specific and JAK/STAT specific.[7] To name a few, STAT5s respond to cellular stimulation by IL-2, IL-3, IL-5, IL-7, GM-CSF, IFN-γ, insulin, erythropoietin, growth hormone, and prolactin.[8]

STAT5a and 5b are involved in different types of cancer (including breast and prostate), inflammatory responses, and allergic reactions.[9] STAT5s also prevent apoptosis in certain cell types and diseases based on their role as transcription factors and the genes they regulate.[10] Due to the transcriptional control provided by the STAT family of proteins, their role in intracellular signaling, and their significance in diseases such as cancer and inflammation, this important signaling pathway is the subject of a number of basic research and pharmacological studies.

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the STAT Kits are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots in the layout shown below. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

1. Phospho-STAT3 (Tyr705)
2. Phospho-STAT4 (Tyr693)
3. Phospho-STAT5a,b (Tyr694)
4. *BSA Blocked*

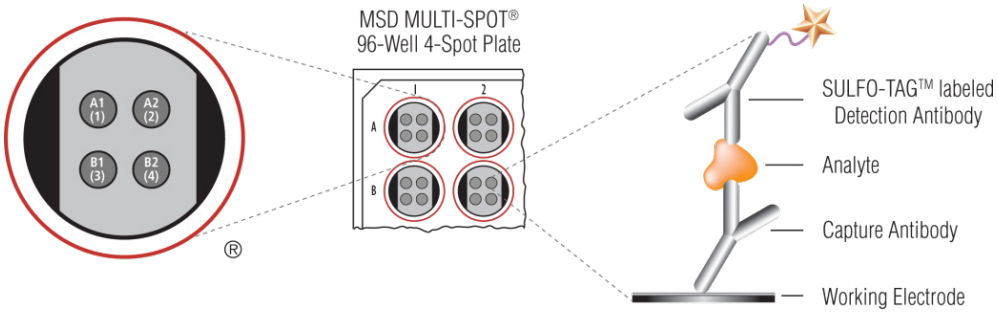


Figure 1. Spot diagram showing placement of analyte capture antibodies for the Phospho-STAT Panel. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

1. STAT
2. *BSA Blocked*
3. *BSA Blocked*
4. *BSA Blocked*

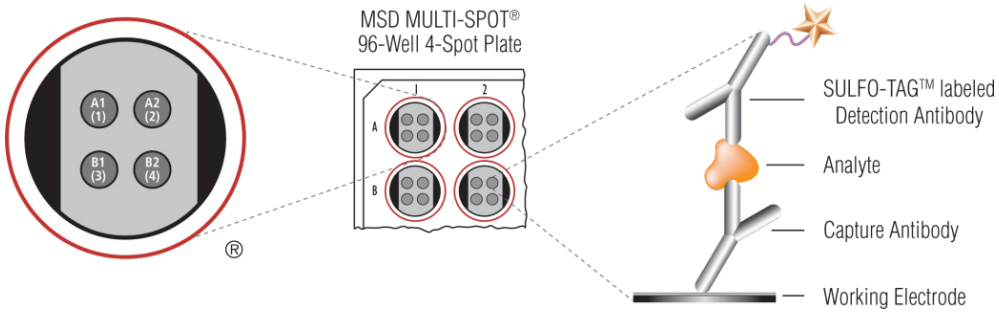


Figure 2. Spot diagram showing placement of analyte capture antibodies for singleplex plates. Singleplex kits are provided on 4-spot plates with analyte capture antibody coated on spot A1. Spots A2, B1, and B2 are BSA blocked.

Kit Components

Reagents Supplied With All Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied					Description
				1-Plate Kit	5-Plate Kit	25 Plate Kit	20-Plate Kit	Base Kit	
Tris Lysis Buffer (1X)	2–8°C	R60TX-3	50 mL	1 bottle	1 bottle	5 bottles			Tris-based lysis buffer for preparing lysates and diluting samples.
		R60TX-2	200 mL				1 bottle		
Tris Wash Buffer (10X)	2–8°C	R61TX-2	200 mL	1 bottle	1 bottle	5 bottles			10X Tris buffered solution with surfactant for washing plates.
		R61TX-1	1000 mL				1 bottle		
Phosphatase Inhibitor I (100X)	2–8°C		0.1 mL	1 vial					Cocktail of serine/threonine protein phosphatase inhibitors
			0.5 mL		1 vial	5 vials			
			2.0 mL				1 vial		
Phosphatase Inhibitor II (100X)	2–8°C		0.1 mL	1 vial					Cocktail of tyrosine protein phosphatase inhibitors
			0.5 mL		1 vial	5 vials			
			2.0 mL				1 vial		
Protease Inhibitor Solution (100X)	2–8°C		0.1 mL	1 vial					Cocktail of protein protease inhibitors
			0.5 mL		1 vial	5 vials			
			2.0 mL				1 vial		
Blocker A (dry powder)	RT	R93BA-4	15 g	1 bottle	1 bottle	1 bottle	1 bottle		Bovine serum albumin, reagent grade pure powder
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles			MSD buffer to catalyze the electrochemiluminescence reaction
		R92TC-2	200 mL				1 bottle	1 bottle	

Additional Reagents Supplied With Phospho-STAT Panel Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied					Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	20-Plate Kit	Base Kit	
Blocker D–M (2%)	≤-10°C		0.2 mL	1 vial					Mouse gamma globulin solution
			0.9 mL						
			1.8 mL		1 vial	5 vials			
Blocker D–R (10%)	≤-10°C		0.05 mL	1 vial					Rabbit gamma globulin solution
			0.2 mL						
			1.0 mL		1 vial	5 vials			

Additional Reagents Supplied With Phospho-STAT4 (Tyr693) Kits and Total STAT4 Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied					Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	20-Plate Kit	Base Kit	
Blocker D–M (2%)	≤-10°C		0.2 mL	1 vial					Mouse gamma globulin solution
			0.9 mL						
			1.8 mL		1 vial	5 vials			
Blocker D–R (10%)	≤-10°C		0.05 mL	1 vial					Rabbit gamma globulin solution
			0.2 mL		1 vial	5 vials			
			1.0 mL						

Kit-Specific Components for Phospho-STAT Panel Kits

Kit Component	Storage	Part #	Size	Quantity Supplied					Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	20-Plate Kit	Base Kit	
Phospho-STAT Panel Plate	2–8°C	N45202A-1	4-spot	1	5	25			96-well plate, foil sealed, with desiccant.
Anti-Phospho-STAT3 (Tyr705) Antibody (50X)	2–8°C	D20SV-2	75 µL	1					SULFO-TAG–conjugated antibody
		D20SV-3	375 µL		1	5			
Anti-Total STAT4 Antibody (50X)	2–8°C	D20OV-2	75 µL	1					SULFO-TAG–conjugated antibody
		D20OV-3	375 µL		1	5			
Anti-Total STAT5a,b (2) Antibody (50X)	2–8°C	D2163-2	75 µL	1					SULFO-TAG–conjugated antibody
		D2163-3	375 µL		1	5			

Kit-Specific Components for Singleplex Kits

Plate Name	Storage	Part #	Size	Quantity Supplied					Kit Name
				1-Plate Kit	5-Plate Kit	25-Plate Kit	20-Plate Kit	Base Kit	
STAT3 Plate	2–8°C	N450SMA-1	4-Spot	1	5	25			Phospho-STAT3 (Tyr705) Total STAT3
Phospho-STAT4 (Tyr693) Plate	2–8°C	N450PAA-1	4-Spot	1	5	25			Phospho-STAT4 (Tyr693)
Phospho-STAT5a,b Plate	2–8°C	N450IGA-1	4-Spot	1	5		20	20	Phospho-STAT5a,b (Tyr694)
Total STAT4 Plate	2–8°C	N450OVA-1	4- Spot	1	5	25			Total STAT4
Total STAT5a,b Plate	2–8°C	N450IHA-1	4- Spot	1	5		20	20	Total STAT5a,b

SULFO-TAG Detection Antibody	Storage	Catalog #	Size	Quantity Supplied					Kit Name
				1-Plate Kit	5-Plate Kit	25-Plate Kit	20-Plate Kit	Base Kit	
Anti-Phospho-STAT3 (Tyr705) Antibody (50X)	2–8°C	D20SV-2	75 µL	1					Phospho-STAT3 (Tyr705)
		D20SV-3	375 µL		1	5			
Anti-Total STAT4 Antibody (50X)	2–8°C	D20OV-2	75 µL	1					Phospho-STAT4 Total STAT4
		D20OV-3	375 µL		1	5			
Anti-Total STAT5a,b (1) Antibody (50X)	2–8°C	D20IG-2	75 µL	1					Phospho-STAT5a,b (Tyr694)
		D20IG-3	375 µL		1		4	4	
Anti- Total STAT3 Antibody (50X)	2–8°C	D20SN-2	75 µL	1					Total STAT3
		D20SN-3	375 µL		1	5			
Anti-Total STAT5ab, Antibody (50X)	2–8°C	D20IH-2	75 µL	1					Total STAT5a,b
		D20IH-3	375 µL		1		4	4	

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm
- ❑ Adhesive plate seals
- ❑ Deionized water

Optional Materials

- ❑ STAT Panel Whole Cell Lysate Set is available for separate purchase from MSD, catalog # C1202-1. The set contains cell lysates from human T-cells that were starved for 30 minutes and then either left untreated or treated for 30 minutes with both IL-12 (10 ng/mL) and IFN-α (1000 U/mL) to stimulate STAT3, STAT4, and STAT5a,b phosphorylation.
- ❑ JAK-STAT Whole Cell Lysate Set is available for separate purchase from MSD, catalog # C10KQ-1. The set contains cell lysates from HeLa cells that were either left untreated or treated with sodium vanadate (1mM) for 4 hours followed by treatment with Oncostatin M (40 ng/mL) to stimulate STAT3 and STAT5a,b phosphorylation.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.
Additional safety information is available in the product safety data sheet, which can be obtained from MSD Customer Service

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific certificate of analysis (C of A).
- Complete lysis buffer should be kept on ice during the experiment.
- Dilute calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting where necessary to avoid introduction of bubbles, and pipette to the bottom corner of empty wells.
- Shaking should be vigorous with a rotary motion between 300 and 1000 rpm.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keeping time intervals consistent between adding read buffer and reading the plate should improve inter-plate precision. Limit the time the plate is incubated with read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the wells.
- When running partial plates, use the sector map in the instrument or software manual to select the wells to be used. Seal the unused portion of the plate with a plate seal to avoid contaminating unused wells. You may adjust volumes proportionally when preparing detection antibody solution. After reading a partial plate, remove fluid, reseal unused sectors, return plate to its original foil pouch with desiccant pack, and seal pouch with tape. Partially used plates may be stored for up to 14 days at 2–8°C.
- Remove plate seals prior to reading the plate.

Reagent Preparation

Prepare Tris Wash Buffer

Dilute the 10X Tris Wash Buffer to 1X as shown below. Tris Wash Buffer (1X) will be used throughout the assay to make additional reagents and to wash plates. Approximately 350 mL per plate are required—more if using an automated plate washer.

For 1 plate, combine:

- ❑ 35 mL of Tris Wash Buffer (10X)
- ❑ 315 mL of deionized water

Excess Tris Wash Buffer may be stored at room temperature in a tightly sealed container.

Prepare Blocker A Solution

For 1 plate, combine:

- ❑ 600 mg Blocker A (dry powder)
- ❑ 20 mL 1X Tris Wash Buffer

Prepare Antibody Dilution Buffer

For the Phospho-STAT Panel Kit, Phospho-STAT4 (Tyr693) Kit, or Total STAT4 Kit, combine for 1 plate:

- ❑ 150 µL 2% Blocker D-M
- ❑ 30 µL 10% Blocker D-R
- ❑ 1 mL of Blocker A solution
- ❑ 1.82 mL of 1X Tris Wash Buffer

For the Phospho-STAT3 (Tyr705) Kit, Total STAT3 Kit, Phospho-STAT5a,b (Tyr694) Kit, or Total STAT5a,b Kit, combine for 1 plate:

- ❑ 1 mL of Blocker A solution
- ❑ 2 mL of 1X Tris Wash Buffer

Prepare Complete Lysis Buffer

Prepare complete lysis buffer just prior to use. The working solution is 1X.

For 1 plate, combine:

- ❑ 50 µL of Protease Inhibitor Solution (100X stock)
- ❑ 50 µL of Phosphatase Inhibitor I (100X stock)
- ❑ 50 µL of Phosphatase Inhibitor II (100X stock)
- ❑ 4.85 mL of 1X Tris Lysis Buffer

Place the complete lysis buffer on ice; it should be ice cold before use.

Prepare and Dilute Samples

Most lysis buffers and sample matrices are compatible with MSD plates, although high concentrations of denaturing reagents should be avoided. Keep SDS and other ionic detergents to a concentration of 0.1% or less in the sample applied to the well. Avoid reducing agents and chaotropes such as DTT >0.1mM and urea >0.1M. Complete lysis buffer may be used to lyse many different cell lines, tumors, and tissues; however, specific sample types may benefit from alternative buffer components. See the Appendix for more detailed information on lysate preparation. Please contact MSD Scientific Support if you have any questions about lysate preparation options.

As a starting point, samples may be diluted to a working concentration of 6.25–800 µg/mL (0.156–20 µg/well with a 25 µL sample volume) using complete lysis buffer as prepared above.

Prepare Control Cell Lysate (Optional)

STAT Panel Whole Cell Lysate Set (catalog # C1202-1) and JAK-STAT Whole Cell Lysate Set (catalog # C10KQ-1) are available for separate purchase from MSD.

Thaw cell lysates on ice and dilute to a working concentration of 1.56–400 µg/mL (0.039–10 µg/well with a 25 µL sample volume) using complete lysis buffer. The lysates can go through an additional freeze–thaw cycle at the stock concentration without significantly affecting analyte levels.

Prepare Detection Antibody Solution

Phospho-STAT Panel Kit

For 1 plate, combine 60 µL of each supplied detection antibody, then add antibody dilution buffer to bring the final volume to 3000 µL.

Singleplex Kits

For 1 plate, add 60 µL of the supplied 50X detection antibody to 2940 µL of antibody dilution buffer.

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

2. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 25 µL of diluted sample or control per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

4. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 150 µL of 1X Read Buffer T to each well. Read plate on MSD instrument. No incubation in read buffer is required before reading the plate; delays may result in lower assay signals for the Phospho-STAT3 (Tyr705) and Total STAT3 assays.

Tested Samples

Whole cell lysates from the STAT Panel Whole Cell Lysate Set (catalog # C1202-1) were titrated and then assayed with the STAT assays. The treatment conditions used for preparing the STAT Panel Whole Cell Lysate Set are outlined below.

- STAT Panel Positive Control Cell Lysates: Human T cells were starved for 30 minutes and then treated for 30 minutes with both IL-12 (10 ng/mL) and IFN-α (1000 U/mL) to stimulate STAT3, STAT4, and STAT5a,b phosphorylation.
- STAT Panel Negative Control Cell Lysates: Human T cells were starved for 30 minutes and then left untreated.

Data from the assays are shown below. Western blot analyses of the whole cell lysates are shown on the following page for comparison.

Phospho-STAT3 (Tyr705)							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	%CV	Ave. Signal	StdDev	%CV	
0	68	16	23.2	74	8	11.4	
0.6	1143	94	8.2	86	8	8.8	13
2.5	5756	1158	11.4	127	27	20.8	45
10.0	29 851	3021	10.1	281	10	3.6	106
Phospho-STAT4 (Tyr693)							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	P/N	Ave. Signal	StdDev	%CV	
0	603	278	46.0	674	23	3.5	
0.6	10 298	243	2.4	839	8	0.9	12
2.5	34 303	1868	5.4	1207	37	3.0	28
10.0	93 057	4053	4.4	3157	133	4.2	29
Phospho-STAT5a,b (Tyr694)							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	%CV	Ave. Signal	StdDev	%CV	
0	67	10	15.2	63	12	18.3	
0.6	5304	187	3.5	413	10	2.5	13
2.5	17 401	243	1.4	1169	30	2.6	15
10.0	56 384	2108	3.7	3758	105	2.8	15
Total STAT3							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	%CV	Ave. Signal	StdDev	%CV	
0	91	2	2.3	107	24	22.5	
0.6	1880	111	5.9	2343	91	3.9	0.8
2.5	9841	352	3.6	16 887	142	0.8	0.6
10.0	62 143	6301	10.1	92 064	8 190	8.9	0.7
Total STAT4							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	%CV	Ave. Signal	StdDev	%CV	
0	85	7	8.2	100	11	10.7	
0.6	83 467	1 586	1.9	152 976	1 682	1.1	0.5
2.5	159 608	2 713	1.7	301 771	1 811	0.6	0.5
10.0	291 583	6 706	2.3	568 487	7 959	1.4	0.5
Total STAT5a,b							
Lysate (µg/well)	Positive			Negative			P/N
	Ave. Signal	StdDev	%CV	Ave. Signal	StdDev	%CV	
0	43	14	32.9	61	7	11.6	
0.6	1175	251	21.4	1296	96	7.4	1.0
2.5	2710	502	18.5	4863	91	1.9	0.6
10.0	11 604	913	7.9	14 934	712	4.8	1.0

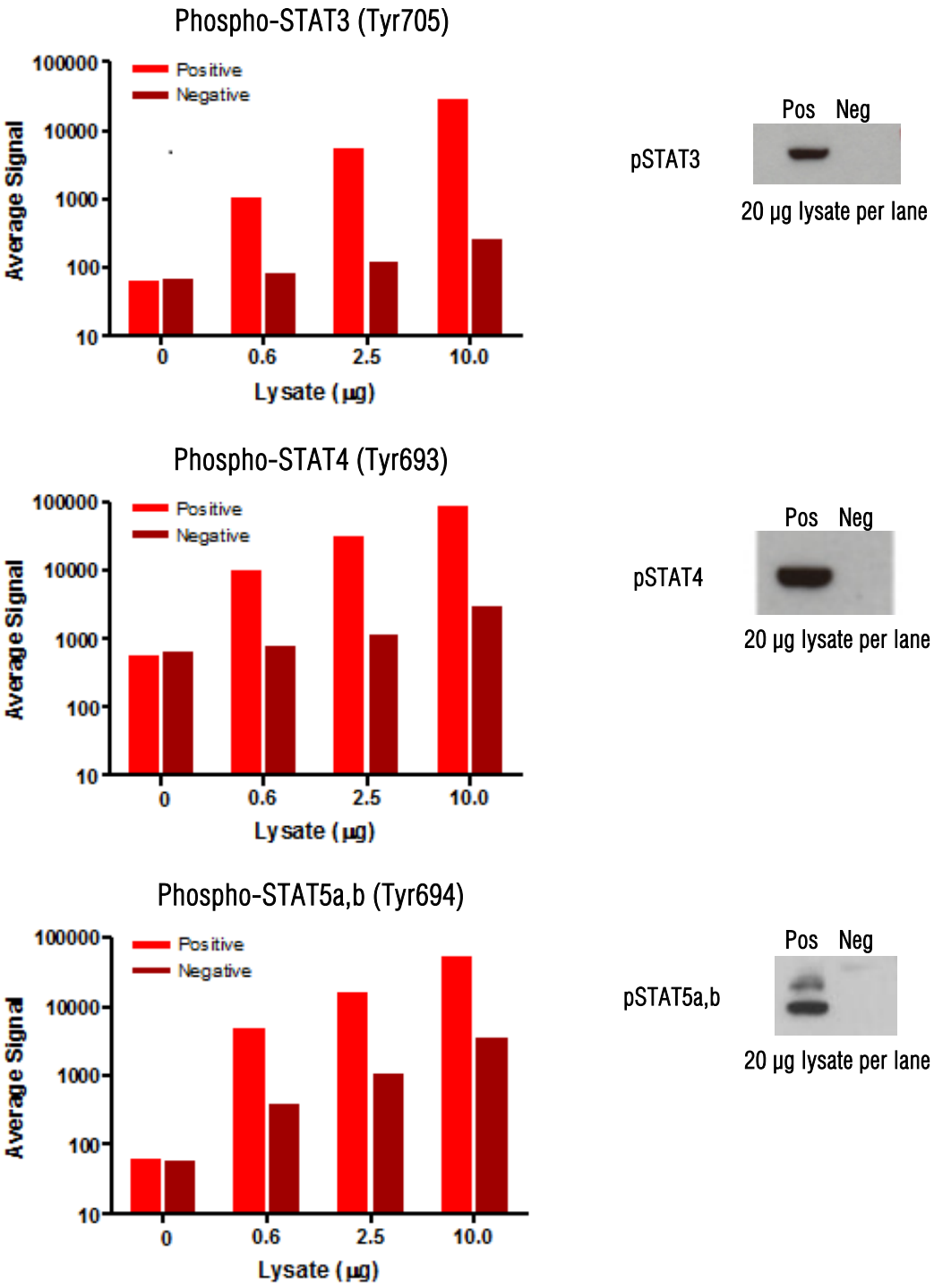


Figure 3: *Sample data generated with Phospho-STAT assays. Increased signals for Phospho-STAT3 (Tyr705), Phospho-STAT4 (Tyr693), and Phospho-STAT5a,b (Tyr694) assays were observed with the titration of lysates from human T-cells that were treated to stimulate STAT3, STAT4, and STAT5a,b phosphorylation (positive) or left untreated (negative). The Phospho-STAT assays provide a quantitative measure of the data obtained with the traditional Western blot test.*

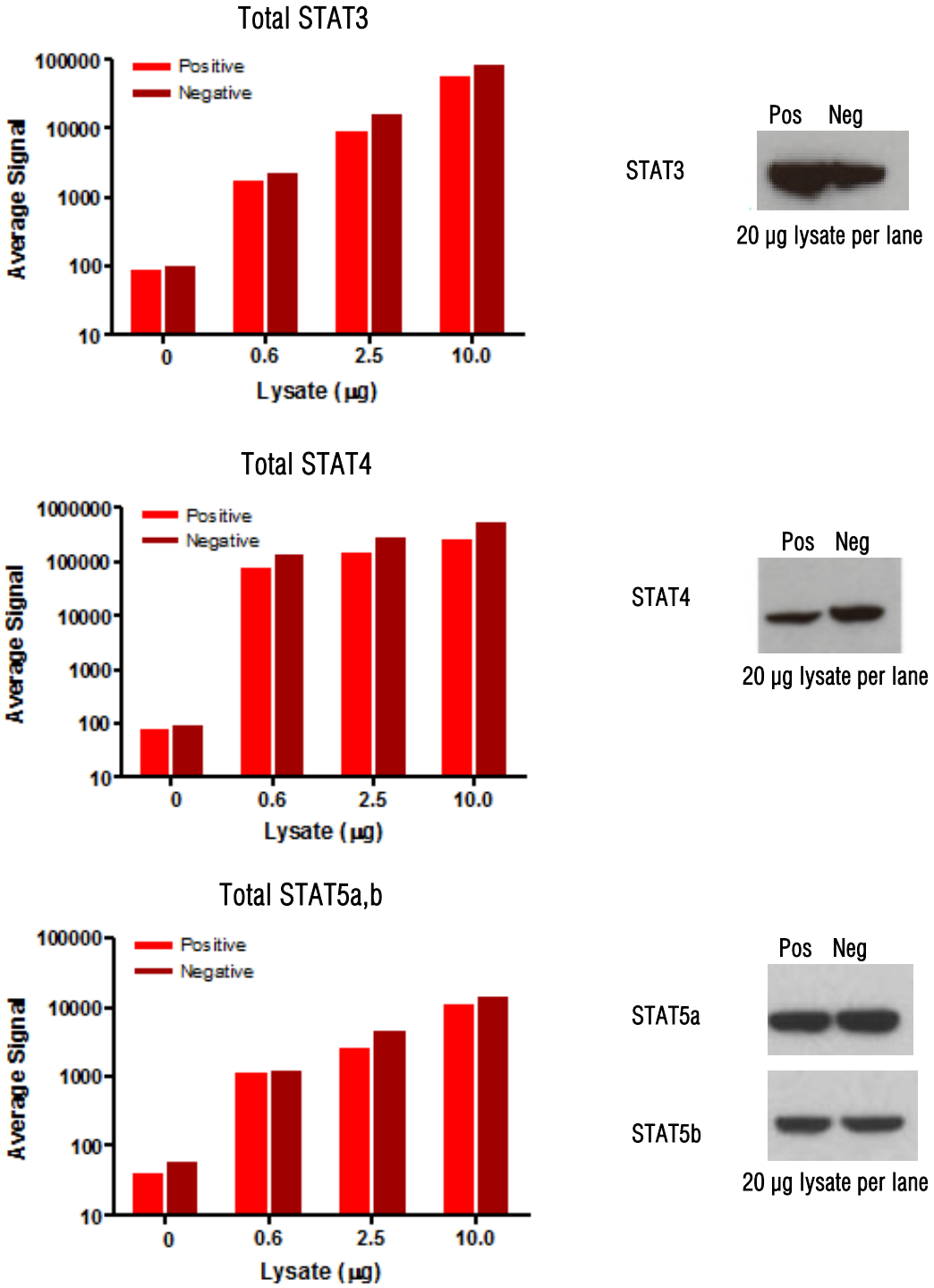


Figure 4: *Sample data generated with Total STAT assays. Increased signals for Total STAT3, Total STAT4, and Total STAT5a,b assays were observed with the titration of lysates from human T-cells that were treated to stimulate STAT3, STAT4, and STAT5a,b phosphorylation (positive) or left untreated (negative). Total STAT levels in the cell model were not significantly affected by the treatment condition since signal levels from the positive and negative lysates are similar. Measuring total STATs in parallel with the phosphorylated form (Figure 3) allows a better understanding of whether a given treatment condition is affecting activation of a protein vs. protein expression levels in the cell model.*

Specificity

Analyte Specificity

No significant level of crossreactivity was observed (<3.1%) for analytes in the Phospho-STAT Panel Kit.

Assay Components

Antibodies

Information on capture and detection antibodies used in the STAT Kits is listed below. The antibodies used in the STAT Kits cross-react with human, mouse, and rat cell lysates.

Phospho-STAT Panel Kit				
Analyte	Phosphospecific Antibody	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Phospho-STAT3 (Tyr705)	Detection	Mouse Monoclonal	Mouse Monoclonal	C
Phospho-STAT4 (Tyr693)	Capture	Mouse Monoclonal	Goat Polyclonal	B
Phospho-STAT5a,b (Tyr694)	Capture	Mouse Monoclonal	STAT5a Mouse Monoclonal STAT5b Rabbit Polyclonal STAT5 Mouse Monoclonal	B

STAT Singleplex Kits				
Analyte	Phosphospecific Antibody	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Phospho-STAT3 (Tyr705)	Detection	Mouse Monoclonal	Mouse Monoclonal	C
Phospho-STAT4 (Tyr693)	Capture	Mouse Monoclonal	Goat Polyclonal	B
Phospho-STAT5a,b (Tyr694)	Capture	Mouse Monoclonal	STAT5a Mouse Monoclonal STAT5b Rabbit Polyclonal	A
Total STAT3	N/A	Mouse Monoclonal	Mouse Monoclonal	A
Total STAT4	N/A	Mouse Monoclonal	Goat Polyclonal	B
Total STAT5a,b	N/A	STAT5a Mouse Monoclonal STAT5b Mouse Monoclonal	Mouse Monoclonal	A

References

1. Wurster AL, et al. The biology of STAT4 and STAT6. Oncogene. 2000;19:2577-2584.
2. Korman BD, et al. STAT4: genetics, mechanisms, and implications for autoimmunity. Curr Allergy Asthma Rep. 2008;8:398-403.
3. Visconti R, et al. Importance of the MKK6/p38 pathway for interleukin-12-induced STAT4 serine phosphorylation and transcriptional activity. Blood. 2000;96:1844-52.
4. Svensson A, et al. STAT4 regulates anti-viral gamma interferon responses and recurrent disease during herpes simplex virus 2 infection. J Virol. 2012;86:9409-15.
5. Cheng X, et al. Adiponectin induces pro-inflammatory programs in human macrophages and CD4+ T Cells. J Biol Chem. 2012;287:36896-904.
6. Clevenger CV. Roles and Regulation of Stat Family Transcription Factors in Human Breast Cancer. Am J Pathol. 2004;165:1449-60.
7. Murray PJ. The JAK-STAT Signaling Pathway: Input and Output Integration J Immunol. 2007;178:2623-9.
8. Liao Z, Lutz J, Nevalainen MT. Transcription factor Stat5a/b as a therapeutic target protein for prostate cancer. Int J Biochem Cell Biol. 2010;42:186-92.
9. Morales JK, Falanga YT, Depcrynski A, Fernando J, Ryan JJ. Mast cell homeostasis and the JAK–STAT pathway. Genes Immun. 2010;11:599-608.
10. 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;21:171-4.

Appendix: Suggested Cell Lysis Protocols

You will need to determine the optimum cell lysis time. Some targets are immediately available for detection. Other targets may require an incubation step at room temperature or on ice with gentle agitation.

Perform all manipulations on ice; keep PBS wash buffer and complete lysis buffer ice cold. Cell concentrations for lysis can range from 0.5 to 5 x 10^7 cells per mL of lysis buffer. Protein yields will vary by cell line. To get your desired final protein concentration, you will need to optimize the number of cells used and the amount of complete lysis buffer added. Depending on the stability of the target in the matrix, you may need additional protease and phosphatase inhibitors in the matrix or diluent. Additionally, specific cell types or targets may benefit from alternative buffer components or techniques, depending upon the particular research application.

Preparation in Culture Flask or Petri Dish

Suspension Cells. Pellet cells by centrifugation at 500g for 3 minutes at 2–8°C. Discard supernatant and wash the pellet once with cold PBS. Pellet cells again, discard supernatant, and resuspend in complete lysis buffer at 1–5 x 10^7 cells per mL. Incubate on ice for 30 minutes. (A shorter incubation time of 15 minutes may be adequate for many targets.) Clear cellular debris from the lysate by centrifuging (≥10 000g) for 10 minutes at 2–8°C. Transfer cleared lysate to a fresh tube and discard the pellet. Determine the protein concentration in the lysate using a detergent-compatible protein assay such as a bicinchoninic acid (BCA) assay. Unused lysates should be aliquoted, quickly frozen in a dry ice-ethanol bath, and stored at ≤-70°C.

Adherent Cells. All volumes given are for cells plated on 15 cm dishes. Remove media from the dish and wash cells once with 5 mL cold PBS. Add 2 mL PBS to each dish, scrape the cells from the surface of the dish, and transfer into 15 mL conical tubes. Pellet the cells by centrifugation at 500g for 3 minutes at 2–8°C. Discard supernatant and resuspend cells in 0.5–2 mL of complete lysis buffer per dish. (Alternatively, cells can be lysed by adding 1–2 mL of complete lysis buffer per 15 cm dish after completely removing the PBS wash buffer.) Incubate on ice for 30 minutes. (A shorter incubation time of 15 minutes may be adequate for many targets.) Clear cellular debris from the lysate by centrifuging (≥10 000g) for 10 minutes at 2–8°C. Transfer cleared lysate to a fresh tube and discard the pellet. Determine protein concentration in the lysate using a detergent-compatible protein assay such as BCA. Unused lysates should be aliquoted, quickly frozen in a dry ice-ethanol bath, and stored at ≤-70°C

Preparation in 96-well Culture Plate

Successful adaptation to a 96-well culture format depends on cell type and target. MSD generally recommends plating concentrations ranging from 1 x 10^4 to 10^5 cells per well; however, the optimal concentrations will vary depending on cell line used.

Suspension Cells. You may lyse many cell types without removing growth medium. For flat bottom plates, design the experiment so that the final suspension cell volume per well is such that a concentrated complete lysis buffer (prepared by the user) can be added to the well to achieve a final 1X lysis buffer concentration in the well. For example, 40 µL of 5X complete lysis buffer added to a well containing 160 µL of cell culture medium would provide a 1X concentration of complete lysis buffer. For conical microwell plates, perform lysis by pelleting the cells, removing most of the growth medium, and adding a constant amount of 1X complete lysis buffer.

Adherent Cells. Plate cells on coated tissue culture plates to reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from the microwell plate to avoid disrupting the cell monolayer. A PBS wash step is not required and can introduce variability as cells may detach during the wash step. Add 100 µL of 1X complete lysis buffer per well. You may modify lysis volume for different cell types or applications. Carefully pipette cell lysate onto prepared assay plate and proceed with assay protocol. Note: It is important to transfer a constant volume and to avoid introducing air bubbles by pipetting too vigorously.

Summary Protocol

STAT Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the STAT assays.

Sample and Reagent Preparation

Prepare Tris Wash Buffer.

Prepare Blocker A solution.

Prepare antibody dilution buffer.

Prepare complete lysis buffer.

Dilute samples to 6.25–800 µg/mL in complete lysis buffer.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in antibody dilution buffer.

Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with shaking for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 25 µL/well of sample (or controls).

Incubate at room temperature with shaking for 1 hour.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with shaking for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 150 µL/well of 1X Read Buffer T.

Analyze plate on MSD instrument.

Plate Diagram