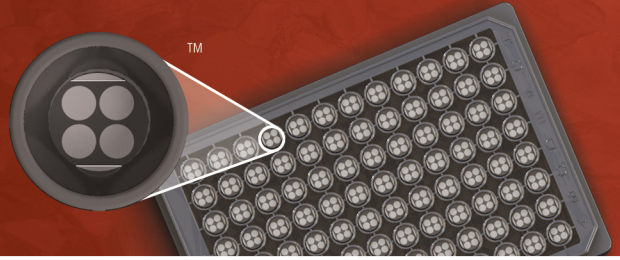
MSD® Akt Signaling Panel II Whole Cell Lysate Kit

For quantitative determination of phosphorylated p70S6K (Thr389), S6RP (Ser240/244), GSK-3β (Ser9), and Akt (Ser473) 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

Akt Signaling Panel II Whole Cell Lysate Kit	
Kit size	
1 plate	K15177D-1
5 plates	K15177D-2
20 plates	K15177D-3

Akt Signaling Panel II Whole Cell Lysate Set	
200 µg	C1177-1

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®

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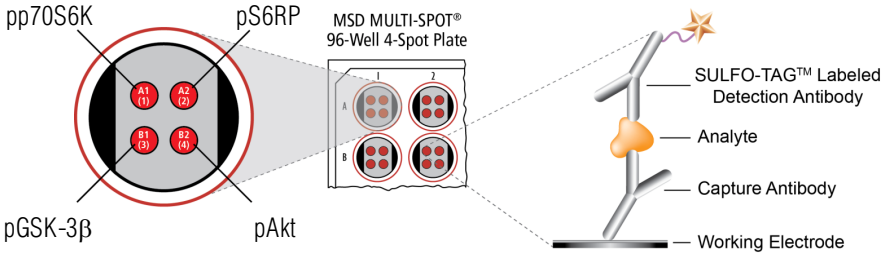

Akt, is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. It affects cell growth by the phosphorylation and inactivation of tuberin (TSC2). Activated Akt promotes growth factor-mediated cell survival by the inhibition of apoptosis.

Ribosomal protein S6 (RPS6 or S6RP), binds to 18s rRNA and becomes part of the small 40s ribosomal subunit. S6RP is phosphorylated on several serine residues by p70S6 kinase (SK1). Ribosomal subunits containing phosphorylated S6 are more likely to be recruited in the formation of polysomes, thus resulting in an upregulation of protein synthesis and cell growth and proliferation.

p70S6K is a serine/threonine kinase that exists in two isoforms within the cell, a 70 kDa cytosolic protein, and an 85 kDa nuclear protein. Activation of p70S6K is linked to the phosphorylation of several serine and threonine residues. p70S6K exerts an effect on translation initiation, cell cycle progression, and cell survival.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that is found in two cellular isoforms - α and β. GSK-3 has diverse cellular effects including involvement in metabolism, embryonic development, and cell survival. GSK-3β has also been implicated in the progression of Alzheimer's disease through the phosphorylation of the microtubule-associated protein tau.

The MSD Akt Signaling Panel II Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Akt Signaling Panel II 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-p70S6K, phospho-S6RP, phospho-GSK-3β, and phospho-Akt antibodies and are shown below for comparison.

Growing MCF-7 cells were treated with IGF-1 (100 nM, 20 minutes) (positive) or LY294002 (50 µM, 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total p70S6K, anti-phospho-S6RP, anti-phospho-GSK-3β, and anti-total Akt antibodies on each of the four spatially distinct electrodes per well. Phosphorylated p70S6K, S6RP, GSK-3β, and Akt were detected with anti-phospho-p70S6K, anti-total S6RP, anti-total GSK-3β, and anti-phospho-Akt antibodies conjugated with MSD SULFO-TAG™ reagent.

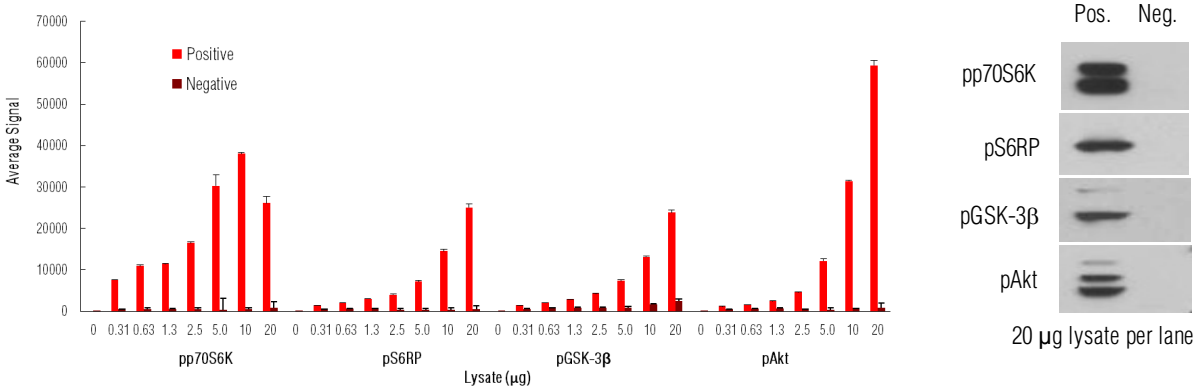

Fig. 1: Sample data generated with MULTI-SPOT Akt Signaling Panel II. Increased signals for phosphorylated forms of p70S6K, S6RP, GSK 3β, and Akt were observed with only Akt Signaling Panel II positive cell lysate. Signals for negative lysate remained low throughout the titration. The Akt Signaling Panel II provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative MCF7 cell lysates using the MULTI-SPOT Akt Signaling Panel II are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pp70S6K	0	84	4	4.2	84	4	4.2	
	0.31	7530	25	0.3	448	42	9.5	17
	0.63	10948	207	1.9	581	11	1.8	19
	1.3	11409	150	1.3	564	4	0.8	20
	2.5	16437	393	2.4	441	77	17.5	37
	5.0	30257	2763	9.1	360	36	10.0	84
	10	38034	362	1.0	490	11	2.3	78
	20	26092	1619	6.2	747	69	9.2	35
pS6RP	0	95	23	24.6	95	23	24.6	
	0.31	1388	24	1.7	497	26	5.3	2.8
	0.63	2013	13	0.6	636	35	5.5	3.2
	1.3	3027	44	1.4	570	33	5.7	5.3
	2.5	3989	213	5.3	381	28	7.4	10
	5.0	7174	264	3.7	344	21	6.0	21
	10	14510	532	3.7	360	23	6.3	40
	20	25047	885	3.5	484	24	5.0	52
pGSK-3β	0	102	18	17.6	102	18	17.6	
	0.31	1403	76	5.4	566	23	4.0	2.5
	0.63	2062	46	2.2	757	33	4.3	2.7
	1.3	2858	71	2.5	850	30	3.5	3.4
	2.5	4229	37	0.9	882	16	1.8	4.8
	5.0	7309	237	3.2	905	6	0.6	8.1
	10	13123	233	1.8	1585	113	7.1	8.3
	20	23860	510	2.1	2452	11	0.4	9.7
pAkt	0	99	10	10.0	99	10	10.0	
	0.31	1146	42	3.7	522	19	3.7	2.2
	0.63	1591	11	0.7	627	1	0.2	2.5
	1.3	2453	151	6.2	631	41	6.5	3.9
	2.5	4551	82	1.8	408	6	1.4	11
	5.0	12105	498	4.1	379	4	1.1	32
	10	31427	169	0.5	429	18	4.1	73
	20	59373	1136	1.9	848	26	3.1	70

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