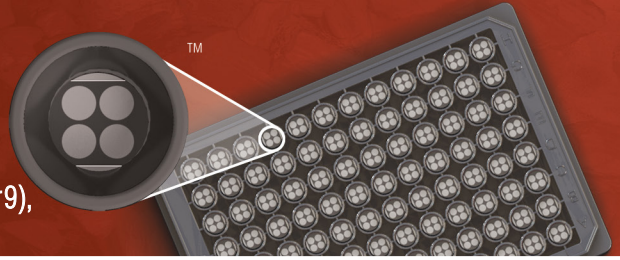
MSD® Akt Signaling Panel Whole Cell Lysate Kit

For quantitative determination of phosphorylated p70S6K (Thr421/Ser424), 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 Whole Cell Lysate Kit	
Kit size	
1 plate	K15115D-1
5 plates	K15115D-2
20 plates	K15115D-3

Akt Signaling Panel Whole Cell Lysate Set	
200 µg	C1115-1

Ordering information

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

Company Address

MESO SCALE DISCOVERY®
A division of
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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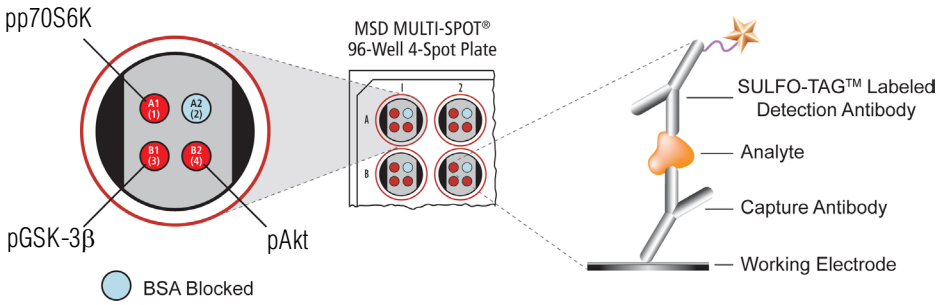

Akt is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. Full activation of Akt1 requires phosphorylation of Thr308 by PDK-1 and subsequent phosphorylation at Ser473 in the hydrophobic motif, which can be mediated by several kinases. In an active form, Akt phosphorylates a wide variety of downstream substrates (i.e., mTOR, p70S6K, and GSK-3β). An anti-apoptotic effect of Akt overexpression has been observed in breast, pancreatic, and ovarian cancer cells. Akt also regulates glycogen synthesis through the inactivation of GSK-3α and GSK-3β.
p70S6K is a serine/threonine kinase that exists in 2 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 residues including Thr229, Thr389, Thr421, Ser411, and Ser424. A diverse array of proteins has been shown to play a role in p70S6K activation including PDK1 and mTOR. In the TORC1 complex (mTOR/Raptor), mTOR signals to its downstream effectors p70S6K/S6RP and 4EBP1/eIF4E to control protein translation.
Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that is found in 2 cellular isoforms - α and β. GSK-3 has diverse cellular effects including involvement in metabolism, embryonic development, and cell survival. The two isoforms are negatively regulated by phosphorylation at Ser21 (GSK-3α) and Ser9 (GSK-3β) mediated by Akt in insulin signal transduction.

The MSD Akt Signaling Panel 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 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-GSK-3β, and phospho-Akt antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells were treated with PMA (200 nM, 15 minutes) (positive) or LY294002 (50 mM) and staurosporine (1 mM) 2.25 hours (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total p70S6K, anti-phospho-GSK-3β, and anti-total Akt antibodies on three of the four spatially distinct electrodes per well. Phosphorylated p70S6K, GSK-3β, and Akt were detected with anti-phospho-p70S6K, anti-total GSK-3β, and anti-phospho-Akt antibodies conjugated with MSD SULFO-TAG™ reagent.

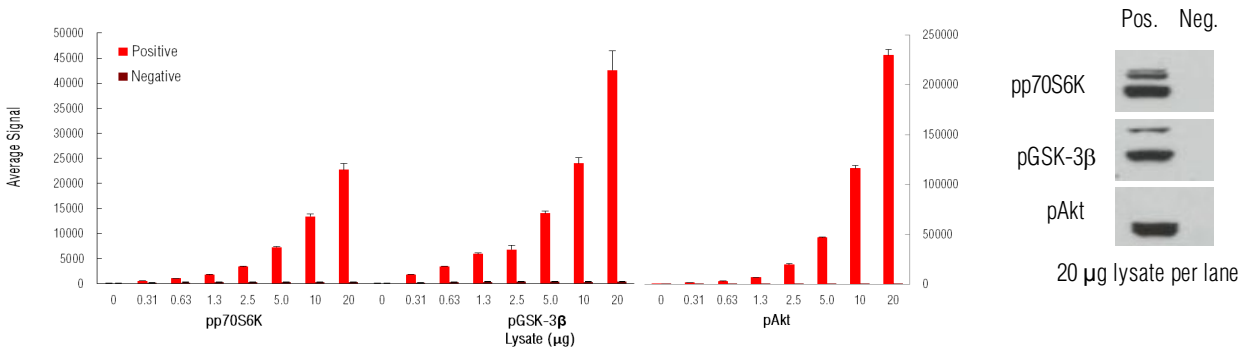

Fig. 1: Sample data generated with MULTI-SPOT Akt Signaling Panel. Increased signals for phosphorylated forms of p70S6K, GSK-3β, and Akt were observed with Akt Signaling Panel positive cell lysate. The Akt Signaling Panel provides a quantitative measure of the data obtained with the traditional Western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

Lysate Titration

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

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pp70S6K	0	116	13	11.4	119	14	11.4	
	0.31	587	3	0.5	217	7	3.1	2.7
	0.63	1068	48	4.5	252	8	3.2	4.2
	1.3	1817	58	3.2	280	9	3.3	6.5
	2.5	3435	30	0.9	306	2	0.8	11
	5.0	7293	136	1.9	310	43	14.0	24
	10	13406	516	3.9	308	8	2.6	44
	20	22757	1230	5.4	306	14	4.5	74
pGSK-3β	0	123	10	8.2	115	5	4.3	
	0.31	1833	30	1.6	243	3	1.2	7.5
	0.63	3442	54	1.6	298	3	1.0	12
	1.3	5965	194	3.3	362	30	8.2	16
	2.5	6852	817	11.9	373	2	0.5	18
	5.0	14072	447	3.2	393	34	8.7	36
	10	24022	1102	4.6	367	8	2.2	65
	20	42582	3916	9.2	393	7	1.8	108
pAkt	0	122	20	16.4	133	7	5.0	
	0.31	1476	24	1.6	243	14	5.7	6.1
	0.63	3079	40	1.3	293	18	6.0	10
	1.3	6848	161	2.4	370	9	2.6	19
	2.5	19833	991	5.0	404	45	11.1	49
	5.0	47091	480	1.0	447	18	4.0	105
	10	116205	2794	2.4	595	17	2.8	195
	20	229927	5742	2.5	893	14	1.5	258

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

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