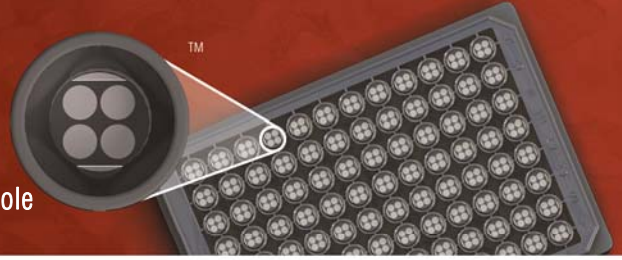
MSD® Akt Signaling (Total Protein) Panel Whole Cell Lysate Kit

For quantitative determination of p70S6K, GSK-3β, and Akt 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 (Total Protein) Panel Whole Cell Lysate Kit	
Kit size	
1 plate	K15133D-1
5 plates	K15133D-2
20 plates	K15133D-3

Akt Signaling (Total Protein) Panel Whole Cell Lysate Set	
200 µg	C1133-1

Ordering information

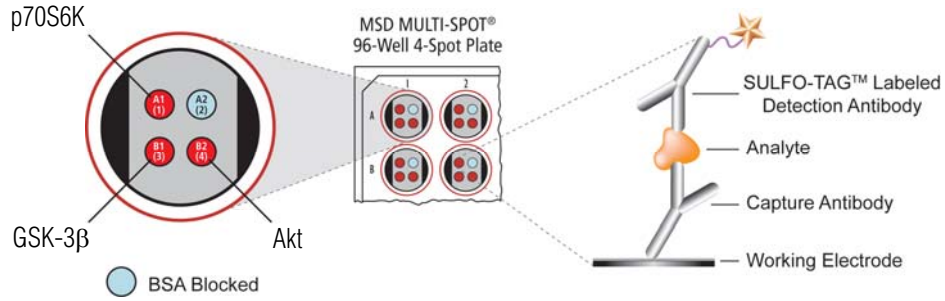
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

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.



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 (Total Protein) 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 (Total Protein) 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 total p70S6K, total GSK-3β, and total Akt antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells were treated with LY294002 (50 µM, 2.5 hours) (negative) or with PMA (200 nM, 15 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total p70S6K, anti-total Akt, and anti-total GSK-3β antibodies on three of the four spatially distinct electrodes per well. p70S6K, Akt, and GSK-3β were detected with anti-total p70S6K, anti-total Akt, and anti-total GSK-3β antibodies conjugated with MSD SULFO-TAG™ reagent.

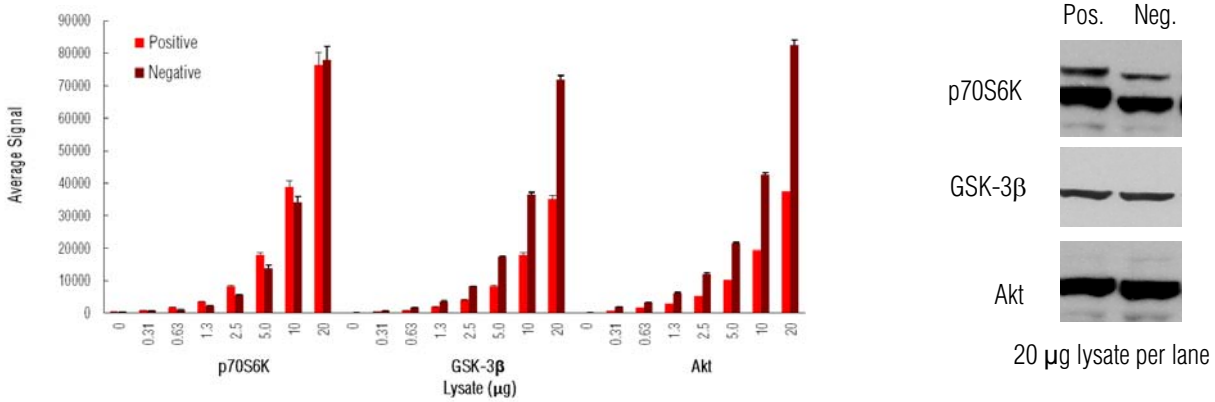


Fig. 1: Sample data generated with MULTI-SPOT Akt Signaling (Total Protein) Panel. Increased signal for p70S6K, GSK-3β, and Akt were observed with both positive and negative cell lysates. The Akt Signaling (Total Protein) Panel provides a quantitative measure of the data obtained with the traditional Western blot.

Spot the Difference™

Lysate Titration

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

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
p70S6K	0	417	14	3.3	419	18	4.2	
	0.31	999	14	1.4	725	66	9.0	1.4
	0.63	1697	51	3.0	1041	42	4.0	1.6
	1.3	3624	81	2.2	2198	103	4.7	1.6
	2.5	8326	274	3.3	5562	270	4.9	1.5
	5.0	17954	822	4.6	13848	483	3.5	1.3
	10	38980	1702	4.4	34141	567	1.7	1.1
	20	76314	4048	5.3	78165	3055	3.9	1.0
GSK-3β	0	146	8	5.2	142	9	6.0	
	0.31	504	10	2.1	899	18	2.0	0.6
	0.63	936	17	1.9	1723	31	1.8	0.5
	1.3	1832	119	6.5	3684	91	2.5	0.5
	2.5	4021	57	1.4	8315	200	2.4	0.5
	5.0	8396	278	3.3	17216	1033	6.0	0.5
	10	17919	691	3.9	36485	1202	3.3	0.5
	20	35041	1152	3.3	71893	2627	3.7	0.5
Akt	0	158	5	2.8	129	21	16.4	
	0.31	876	56	6.4	1784	75	4.2	0.5
	0.63	1596	73	4.6	3315	179	5.4	0.5
	1.3	2859	29	1.0	6245	400	6.4	0.5
	2.5	5285	311	5.9	12009	199	1.7	0.4
	5.0	10161	113	1.1	21572	459	2.1	0.5
	10	19457	811	4.2	42452	1324	3.1	0.5
	20	37367	1596	4.3	82518	3243	3.9	0.5

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