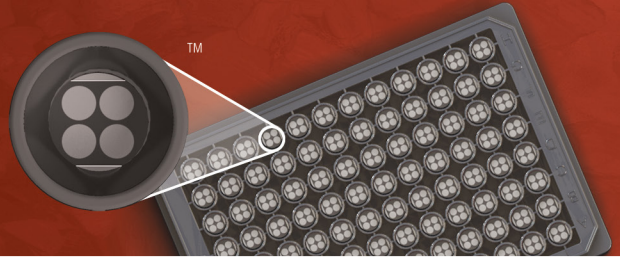
MSD® Phospho(Ser473)/Total Akt Assay Whole Cell Lysate Kit

For quantitative determination 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

Phospho(Ser473)/Total Akt Assay - Whole Cell Lysate Kit	
Kit size	
1 plate	K15100D-1
5 plates	K15100D-2
20 plates	K15100D-3

Phospho-Akt (Ser473) Whole Cell Lysate Set	
200 µg	C11CA-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
Meso Scale Diagnostics, LLC.
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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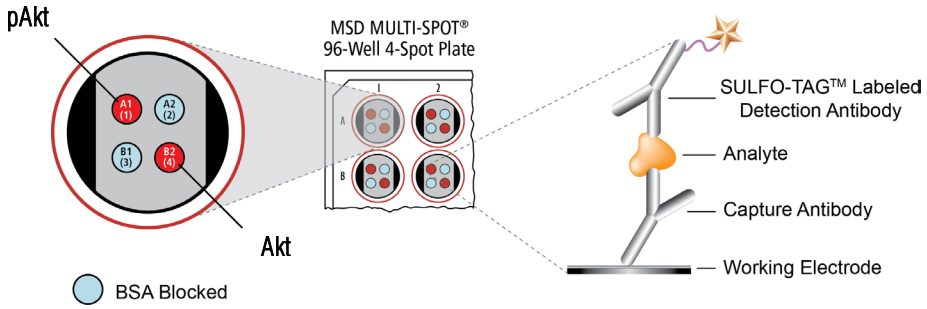

Akt, also known as protein kinase B (PKB) or Rac, is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. The three mammalian isoforms, Akt1, Akt2, and Akt3, contain an amino-terminal pleckstrin homology (PH) domain, central catalytic domain, and carboxy-terminal regulatory region. The PH domain of Akt binds to lipid products generated by phosphoinositide 3-kinase (PI3K). This binding event results in the translocation of Akt to the plasma membrane. The outcome is a conformational change and activation of Akt by phosphorylation on Thr308 and Ser473 by 3-phosphoinositide-dependent kinase-1 (PDK1) and possibly by other additional kinases. In its active form, Akt phosphorylates a wide variety of targets. Akt affects cell growth by the phosphorylation and inactivation of tuberin (TSC2), an inhibitor of mTOR. Activated Akt promotes growth factor-mediated cell survival by the inhibition of apoptosis through several pathways, including the inactivation of BAD, Caspase-9, IKKα, and the forkhead transcription factors. Anti-apoptotic effect of Akt overexpression has been observed in breast, pancreatic, and ovarian cancer cells.

The MSD Phospho(Ser473)/Total Akt Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Ser473)/Total Akt 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-Akt (Ser473) and total Akt antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM; 2.25 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-Akt antibody and anti-total Akt antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total Akt were detected with anti-total Akt antibody labeled with MSD SULFO-TAG™ reagent.

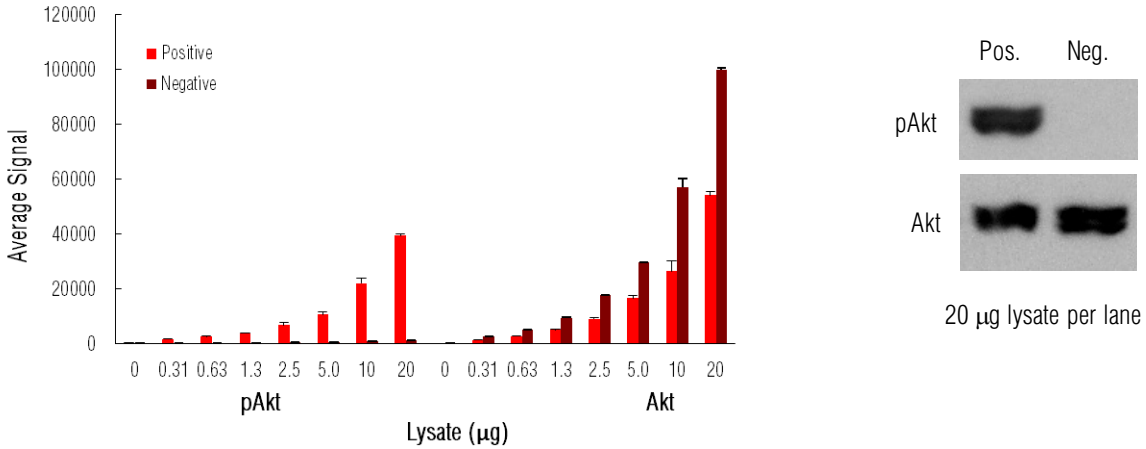

Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Ser473)/Total Akt Assay. Increased signal for phosphorylated Akt was observed with only pAkt positive cell lysate. Total Akt signal increased throughout the titration of both pAkt positive and negative cell lysates. The Phospho(Ser473)/Total Akt Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pAkt positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Ser473)/Total Akt Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pAkt	0	368	122	33.2	286	54	18.7	
	0.31	1659	118	7.1	304	46	15.0	5.5
	0.63	2598	297	11.4	396	23	5.9	6.6
	1.3	3908	139	3.6	392	14	3.4	10
	2.5	6875	935	13.6	543	18	3.2	13
	5.0	10724	933	8.7	676	39	5.8	16
	10	22151	1760	7.9	849	113	13.3	26
	20	39455	761	1.9	1239	103	8.3	32
Akt	0	109	10	8.8	101	8	8.0	
	0.31	1462	57	3.9	2737	107	3.9	0.5
	0.63	2843	131	4.6	5092	230	4.5	0.6
	1.3	5070	300	5.9	9407	239	2.5	0.5
	2.5	9114	337	3.7	17709	86	0.5	0.5
	5.0	16738	811	4.8	29691	51	0.2	0.6
	10	26538	3884	14.6	57072	3114	5.5	0.5
	20	54182	1457	2.7	99970	593	0.6	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

References using MSD's platform for the measurement of phosphoproteins

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2. Grimshaw KM, Hunter LJ, Yap TA, Heaton SP, Walton MI, Woodhead SJ, Fazal L, Reule M, Davies TG, Seavers LC, Lock V, Lyons JF, Thompson NT, Workman P, Garrett MD. AT7867 is a potent and oral inhibitor of AKT and p70 S6 kinase that induces pharmacodynamic changes and inhibits human tumor xenograft growth. Mol Cancer Ther. 2010 May;9(5):1100-10. Epub 2010 Apr 27.
3. Gowan SM, Hardcastle A, Hallsworth AE, Valenti MR, Hunter LJ, de Haven Brandon AK, Garrett MD, Raynaud F, Workman P, Aherne W, Eccles SA. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol. 2007 Jun;5(3):391-401.