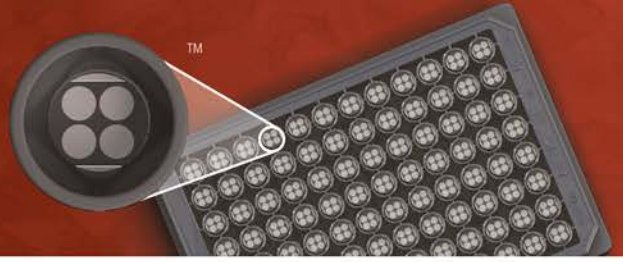
MSD® Phospho-Akt (Thr308) Assay Whole Cell Lysate Kit

For quantitative determination in human and mouse whole cell lysate samples



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Phospho-Akt (Thr308) Assay Whole Cell Lysate Kit	
Kit size	
1 plate	K151DYD-1
5 plates	K151DYD-2
20 plates	K151DYD-3

Phospho-Akt (Thr308) Whole Cell Lysate Set	
200 µg	C11DY-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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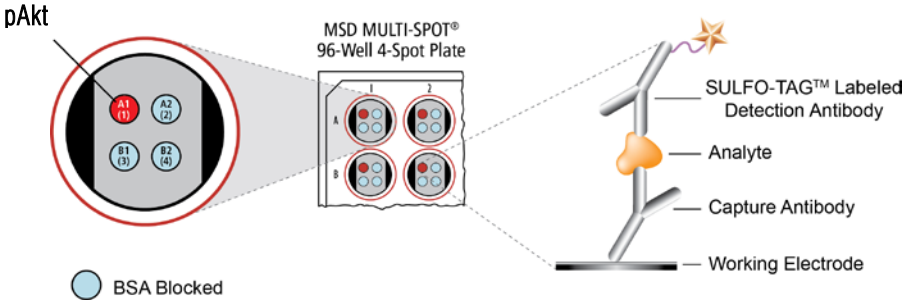
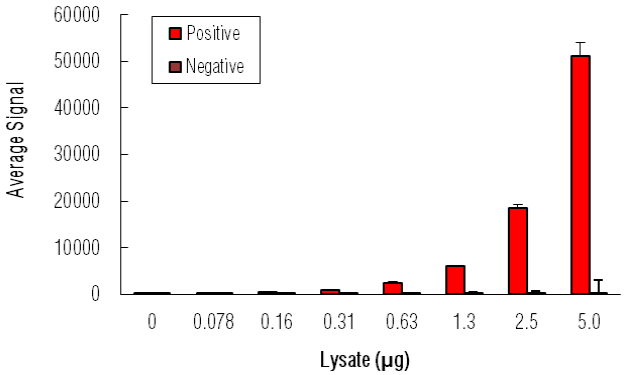


Akt, also known as protein kinase B (PKB) or Rac, is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. The three mammalian isoforms, Akt1, Akt2, and Akt3, contain an amino-terminal pleckstrin 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-Akt (Thr308) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-Akt (Thr308) 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 (Thr308) and total Akt antibodies and are shown below for comparison.
Confluent NIH3T3 cells (negative) were treated with PDGF (100 ng/mL; 5 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total Akt antibody on one of the four spatially distinct electrodes per well. Phosphorylated Akt was detected with an anti-phosphorylated Akt (Thr308) antibody labeled with MSD SULFO-TAG™ reagent.



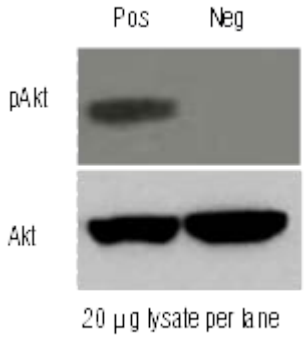


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-Akt (Thr308) Assay. Increased signal is observed with the titration of pAkt positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-Akt (Thr308) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pAkt positive and negative NIH3T3 cell lysates using the MULTI-ARRAY Phospho-Akt (Thr308) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	104	5	4.8	116	8	6.7	
0.078	269	22	8.2	115	1	1.2	2.3
0.16	447	32	7.1	121	0	0.0	3.7
0.31	840	23	2.8	131	6	4.3	6.4
0.63	2534	147	5.8	170	6	3.8	15
1.3	5977	286	4.8	192	8	4.1	31
2.5	18605	580	3.1	215	5	2.3	87
5.0	51199	2886	5.6	253	13	5.0	202

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample volumes 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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