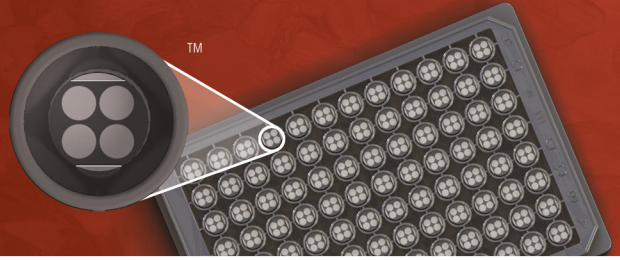
MSD® Total Akt Assay Whole Cell Lysate Kit

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

Total Akt Assay Whole Cell Lysate Kit	
Kit size	
1 plate	K151CBD-1
5 plates	K151CBD-2
20 plates	K151CBD-3

Phospho-Akt (Ser473) Whole Cell Lysate Set	
200 µg	C11CA-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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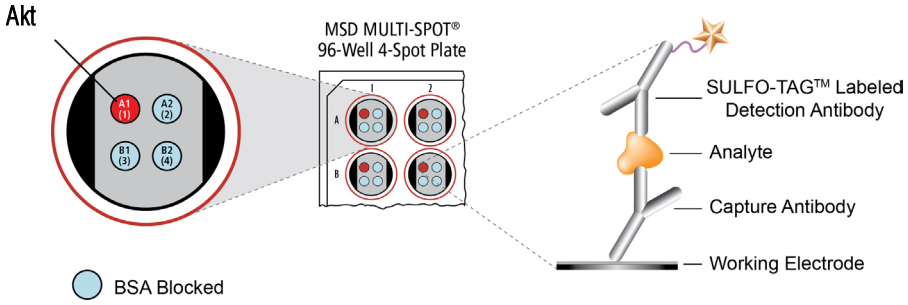

Akt, also known as protein kinase B (PKB) or Rac, is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. The three mammalian isoforms, Akt1, Akt2, and Akt3, contain an amino-terminal pleckstrin 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 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 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-total Akt antibody on one of the four spatially distinct electrodes per well. Total Akt was detected with anti-total Akt antibody labeled with MSD SULFO-TAG™ reagent.

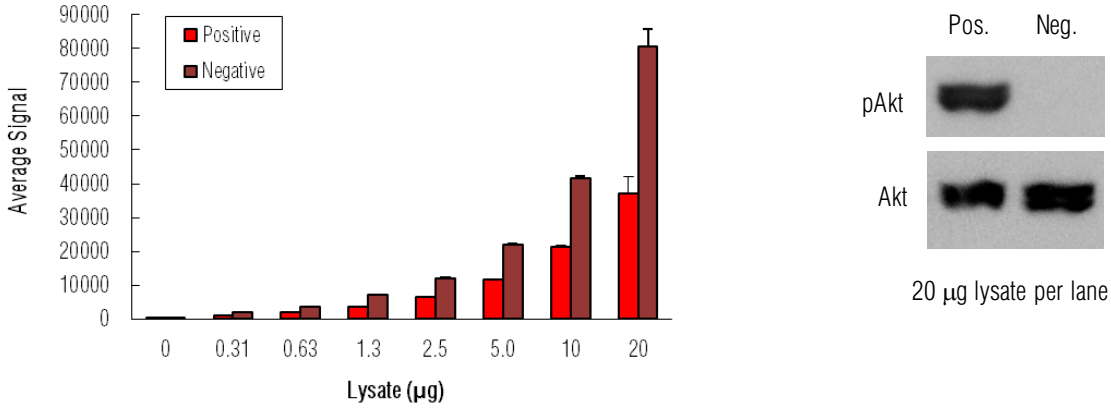

Fig. 1: Sample data generated with the MULTI-ARRAY® Total Akt Assay. Increased signal is observed with the titration of both pAkt positive and pAkt negative cell lysates. The Total Akt Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Spot the Difference™

Lysate Titration

Data for pAkt positive and negative Jurkat cell lysates using the MULTI-ARRAY Total Akt Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	84	9	11.3	93	7	7.7	
0.31	1123	31	2.8	1896	81	4.3	0.6
0.63	2015	94	4.7	3547	152	4.3	0.6
1.3	3714	240	6.5	7040	243	3.4	0.5
2.5	6654	178	2.7	12013	920	7.7	0.6
5.0	11720	227	1.9	22067	806	3.7	0.5
10	21204	564	2.7	41635	1278	3.1	0.5
20	36993	5101	13.8	80673	2330	2.9	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

References using MSD's platform for the measurement of phosphoproteins

1. Edgar KA, Wallin JJ, Berry M, Lee LB, Prior WW, Sampath D, Friedman LS, Belvin M. Isoform-specific phosphoinositide 3-kinase inhibitors exert distinct effects in solid tumors. Cancer Res. 2010 Feb 1;70(3):1164-72. Epub 2010 Jan 26.
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. Paoloni MC, Mazcko C, Fox E, Fan T, Lana S, Kisseberth W, Vail DM, Nuckolls K, Osborne T, Yalkowsy S, Gustafson D, Yu Y, Cao L, Khanna C. Rapamycin pharmacokinetic and pharmacodynamic relationships in osteosarcoma: a comparative oncology study in dogs. PLoS One. 2010 Jun 8;5(6):e11013.
4. Sutherlin DP, Sampath D, Berry M, Castanedo G, Chang Z, Chuckowree I, Dotson J, Folkes A, Friedman L, Goldsmith R, Heffron T, Lee L, Lesnick J, Lewis C, Mathieu S, Nonomiya J, Olivero A, Pang J, Prior WW, Salphati L, Sideris S, Tian Q, Tsui V, Wan NC, Wang S, Wiesmann C, Wong S, Zhu BY. Discovery of (thienopyrimidin-2-yl) aminopyrimidines as potent, selective, and orally available pan-PI3-kinase and dual pan-PI3-kinase/mTOR inhibitors for the treatment of cancer. J Med Chem. 2010 Feb 11;53(3):1086-97.
5. 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.