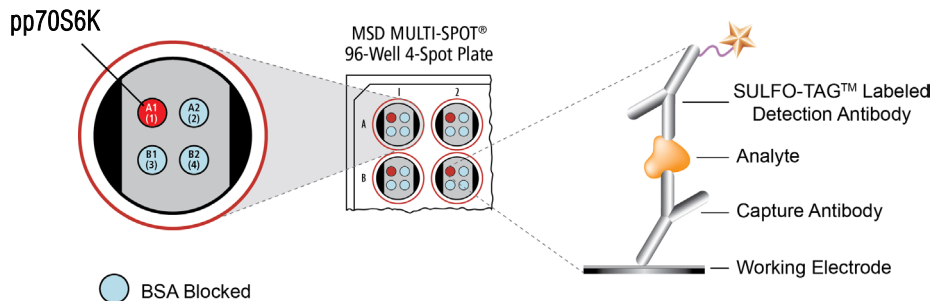
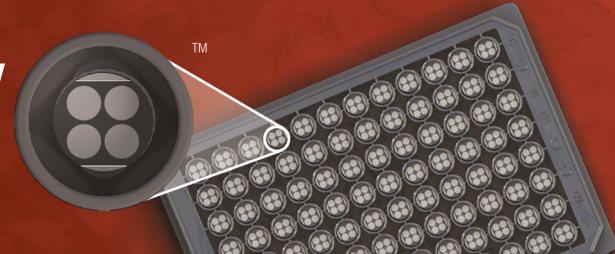


MSD® Phospho-p70S6K (Thr421/Ser424) Assay Whole Cell Lysate Kit

For quantitative determination in human, mouse, and rat whole cell lysate samples



The serine/threonine kinase **p70S6K** exists in two isoforms within the cell, a 70 kDa cytosolic protein, and an 85 kDa nuclear protein. The small ribosomal protein S6 (of the 40S subunit) is phosphorylated by active p70S6K on five serine residues. Activation of p70S6K is linked to the phosphorylation of several serine and threonine residues including threonines at positions 229, 389, and 421, and serines at positions 411 and 424. A diverse array of proteins have been shown to play a role in p70S6K activation including PDK1, the G proteins Cdc42 and Rac1, mTOR, and the c-Raf/MEK/ERK pathway. These effectors are activated upstream by insulin, amino acids, and growth factors. In response, p70S6K exerts an effect on translation initiation, cell cycle progression, and cell survival.

The MSD Phospho-p70S6K (Thr421/Ser424) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-p70S6K (Thr421/Ser424) 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-p70S6K (Thr421/Ser424) and total p70S6K antibodies and are shown below for comparison. Growing HEK293 cells were treated with rapamycin (1 μ M; 3 hours) (negative). Serum deprived HEK293 cells were treated with PMA (1 μ M; 30 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total p70S6K antibody on one of the four spatially distinct electrodes per well. Phosphorylated p70S6K was detected with anti-phospho-p70S6K (Thr421/Ser424) antibody conjugated with MSD SULFO-TAG™ reagent.

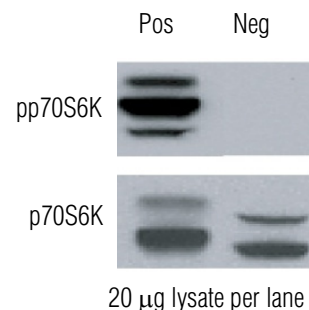
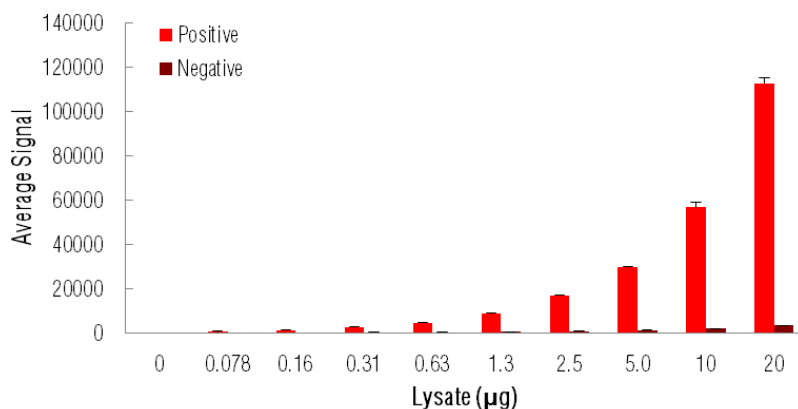


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-p70S6K (Thr421/Ser424) Assay. Increased signal is observed with the titration of pp70S6K positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-p70S6K (Thr421/Ser424) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

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

Catalog Numbers

Phospho-p70S6K
(Thr421/Ser424) Assay:
Whole Cell Lysate Kit

Kit size

1 plate K150DCD-1

5 plates K150DCD-2

20 plates K150DCD-3

Phospho-p70S6K
(Thr421/Ser424) Whole Cell
Lysate Set

200 μ g C11DC-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.

MSD Phosphoprotein Assays

Lysate Titration

Data for pp70S6K positive and negative HEK293 cell lysates using the MULTI-ARRAY Phospho-p70S6K (Thr421/Ser424) Assay are presented below.

Lysate (μ g)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	158	10	6.3	158	10	6.3	
0.078	732	42	5.7	208	32	15.3	3.5
0.16	1347	35	2.6	236	6	2.7	5.7
0.31	2715	96	3.5	273	30	11.2	10
0.63	4702	218	4.6	336	44	13.0	14
1.3	9008	69	0.8	547	33	6.1	16
2.5	17067	187	1.1	810	8	1.0	21
5.0	29978	206	0.7	1269	117	9.2	24
10	56732	2544	4.5	1995	35	1.7	28
20	112924	2321	2.1	3449	132	3.8	33

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 μ g/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. Beltran PJ, Chung YA, Moody G, Mitchell P, Cajulis E, Vonderfecht S, Kendall R, Radinsky R, Calzone FJ. Efficacy of ganitumab (AMG 479), alone and in combination with rapamycin, in Ewing's and osteogenic sarcoma models. J Pharmacol Exp Ther. 2011 Jun;337(3):644-54. Epub 2011 Mar 8.
2. Guillard S, Clarke PA, Te Poele R, Mohri Z, Bjerke L, Valenti M, Raynaud F, Eccles SA, Workman P. Molecular pharmacology of phosphatidylinositol 3-kinase inhibition in human glioma. Cell Cycle. 2009 Feb 1;8(3):443-53. Epub 2009 Feb 16.
3. Raynaud FI, Eccles SA, Patel S, Alix S, Box G, Chuckowree I, Folkes A, Gowan S, De Haven Brandon A, Di Stefano F, Hayes A, Henley AT, Lensun L, Pergl-Wilson G, Robson A, Saghir N, Zhyvoloup A, McDonald E, Sheldrake P, Shuttleworth S, Valenti M, Wan NC, Clarke PA, Workman P. Biological properties of potent inhibitors of class I phosphatidylinositide 3-kinases: from PI-103 through PI-540, PI-620 to the oral agent GDC-0941. Mol Cancer Ther. 2009 Jul;8(7):1725-38. Epub 2009 Jul 7.
4. 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.

MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, WWW.MESOSCALE.COM, MSD, MSD (DESIGN), DISCOVERY WORKBENCH, QUICKPLEX, MULTI-ARRAY, MULTI-SPOT, SULFO-TAG, SECTOR, SECTOR HTS, SECTOR PR, 4-SPOT (DESIGN) and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC.
© 2011 Meso Scale Diagnostics, LLC. All rights reserved.

For Research Use Only. Not for use in diagnostic procedures.

