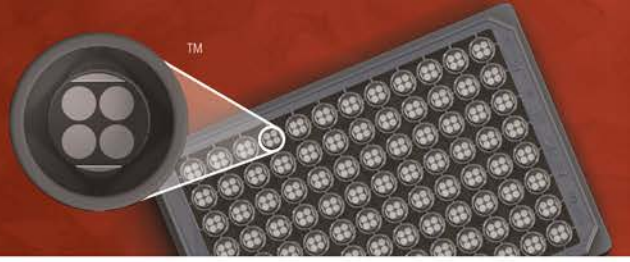
MSD® Total p70S6K Assay Whole Cell Lysate Kit

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 p70S6K Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150DDD-1
5 plates	K150DDD-2
20 plates	K150DDD-3

Phospho-p70S6K (Thr389) Whole Cell Lysate Set	
200 µg	C11DN-1

Phospho-p70S6K (Thr421/Ser424) Whole Cell Lysate Set	
200 µg	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®
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Meso Scale Diagnostics, LLC.
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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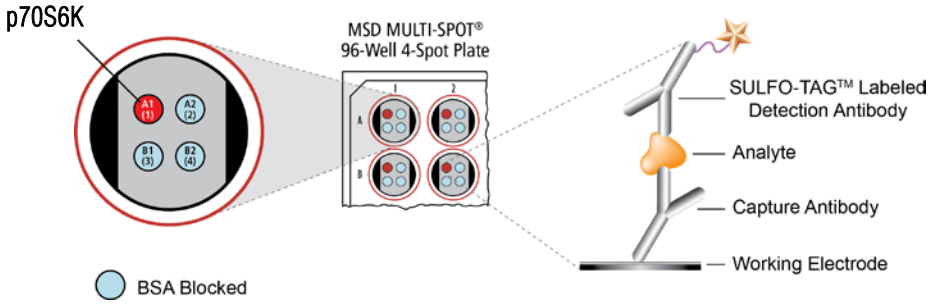

The serine/threonine kinase **p70S6K** exists in two isoforms within the cell, a 70 kDa cytosolic protein, and an 85 kDa nuclear protein. The small ribosomal protein S6 (of the 40S subunit) is phosphorylated by active p70S6K on five serine residues. Activation of p70S6K is linked to the phosphorylation 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 Total p70S6K Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Total p70S6K 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 (Thr389), phospho-p70S6K (Thr421/Ser424), and total p70S6K antibodies and are shown below for comparison. Growing HEK293 cells were treated with rapamycin (1 µM; 3 hours) (negative) or Calyculin A (50 nM, 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. Total p70S6K was detected with anti-Total p70S6K antibody conjugated with MSD SULFO-TAG™ reagent.

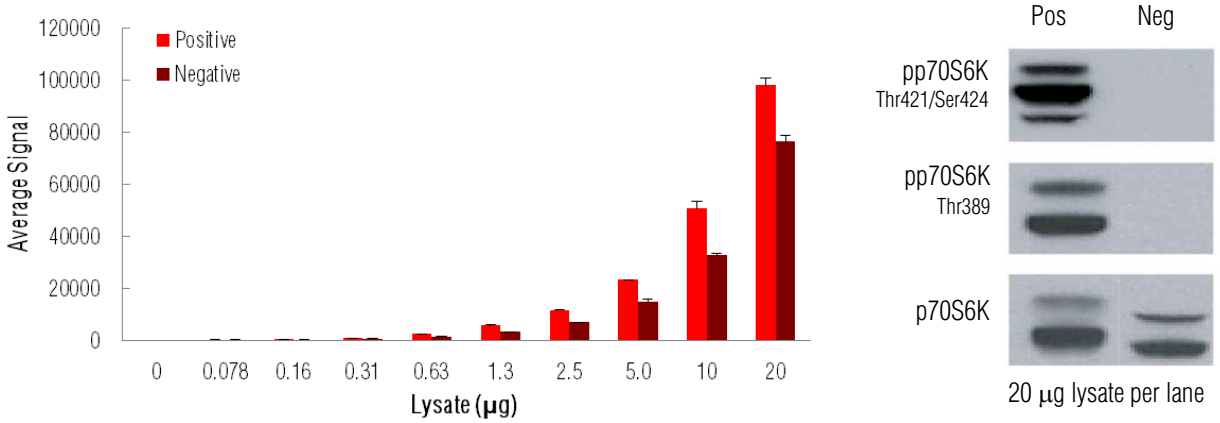

Fig. 1: Sample data generated with the MULTI-ARRAY® Total p70S6K Assay. Increased signal is observed with the titration of both pp70S6K positive and negative cell lysates. The Total p70S6K Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Spot the Difference™

Lysate Titration

Data for pp70S6K positive and negative HEK293 cell lysates using the MULTI-ARRAY Total p70S6K Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	134	9	6.5	134	9	6.5	
0.078	304	11	3.7	255	4	1.7	1.2
0.16	532	10	1.9	382	12	3.2	1.4
0.31	1042	6	0.5	682	38	5.6	1.5
0.63	2527	33	1.3	1459	25	1.7	1.7
1.3	5779	205	3.5	3386	58	1.7	1.7
2.5	11725	284	2.4	6990	146	2.1	1.7
5.0	23393	96	0.4	15017	745	5.0	1.6
10	50678	2826	5.6	32738	728	2.2	1.5
20	98081	2865	2.9	76592	2439	3.2	1.3

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. 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.