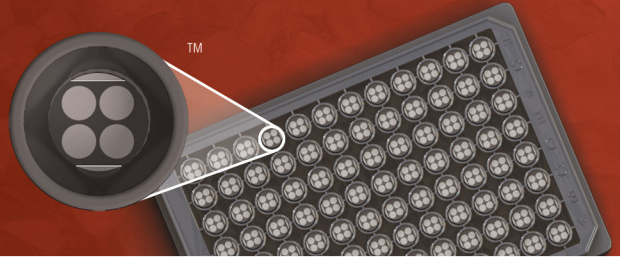
MSD® Phospho(Thr421/Ser424)/ Total p70S6K Assay: Whole Cell Lysate Kit

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(Thr421/Ser424)/ Total p70S6K Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K15114D-1
5 plates	K15114D-2
20 plates	K15114D-3

Phospho-p70S6K (Thr421/Ser424) Whole Cell Lysate Set	
200 µg	C11DC-1

Ordering information

MSD Customer Service
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Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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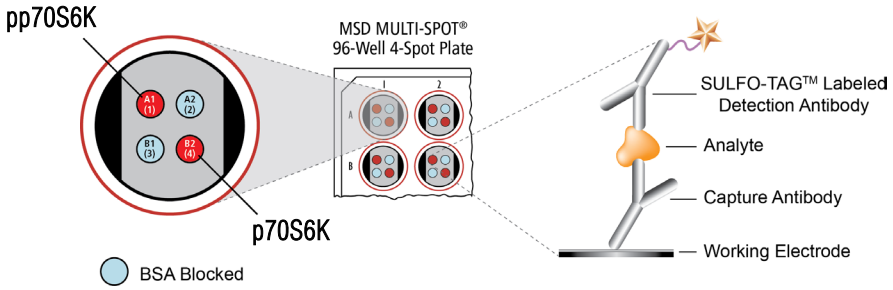

The serine/threonine kinase **p70S6K** exists in two isoforms within the cell, a 70 kDa cytosolic protein, and an 85 kDa nuclear protein. The small ribosomal protein S6 (of the 40S subunit) is phosphorylated by active p70S6K on five serine residues. Activation of p70S6K is linked to the phosphorylation 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 Phospho(Thr421/Ser424)/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 Phospho(Thr421/Ser424)/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 (Thr421/Ser424) and total p70S6K antibodies and are shown below for comparison.
Growing HEK293 cells were treated with rapamycin (1 µM; 3 hours) (negative). Serum deprived HEK293 cells were treated with PMA (1 µM; 30 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-p70S6K (Thr421/Ser424) antibody and anti-total p70S6K antibody on spatially distinct electrodes within a well. Phosphorylated and total p70S6K were detected with anti-total p70S6K antibody conjugated with MSD SULFO-TAG™ reagent.

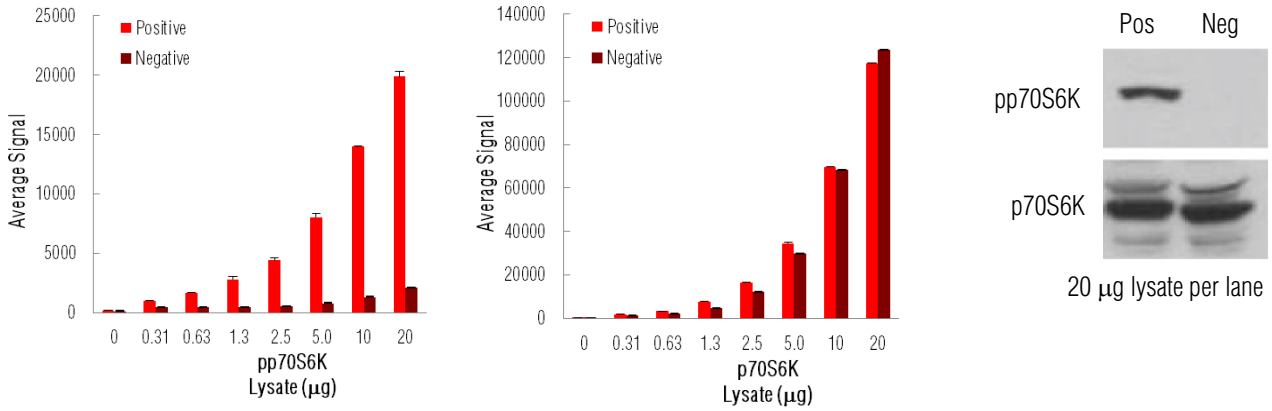

Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Thr421/Ser424)/Total p70S6K Assay. Increased signal for phosphorylated p70S6K was observed with pp70S6K positive cell lysate. Total p70S6K signal increased throughout the titration of both pp70S6K positive and negative cell lysates. The Phospho(Thr421/Ser424)/Total p70S6K Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

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

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pp70S6K	0	155	42	27.0	102	13	12.5	
	0.31	1013	18	1.7	418	24	5.8	2.4
	0.63	1657	4	0.3	463	5	1.1	3.6
	1.3	2790	247	8.8	447	27	6.0	6.2
	2.5	4400	206	4.7	509	51	10.0	8.6
	5.0	8035	288	3.6	762	65	8.5	11
	10	13969	98	0.7	1263	75	5.9	11
	20	19887	424	2.1	2074	13	0.6	9.6
p70S6K	0	155	7	4.6	164	28	16.9	
	0.31	1760	103	5.8	1078	36	3.3	1.6
	0.63	3358	226	6.7	2111	30	1.4	1.6
	1.3	7617	204	2.7	4556	37	0.8	1.7
	2.5	16539	92	0.6	12167	96	0.8	1.4
	5.0	34534	175	0.5	29601	699	2.4	1.2
	10	69677	3853	5.5	68178	2777	4.1	1.0
	20	117313	180	0.2	123826	1,161	0.9	0.9

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. 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.

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