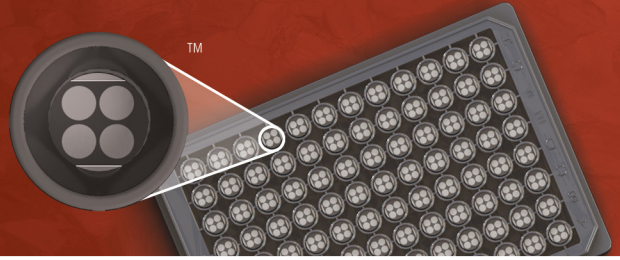
MSD® Phospho(Ser240/244)/Total S6RP Assay Whole Cell Lysate Kit

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

Phospho(Ser240/244)/Total S6RP: Whole Cell Lysate Kit	
Kit size	
1 plate	K15139D-1
5 plates	K15139D-2
20 plates	K15139D-3

Phospho-S6RP Whole Cell Lysate Set	
200 µg	C11DF-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
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Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
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Gaithersburg, MD 20877 USA

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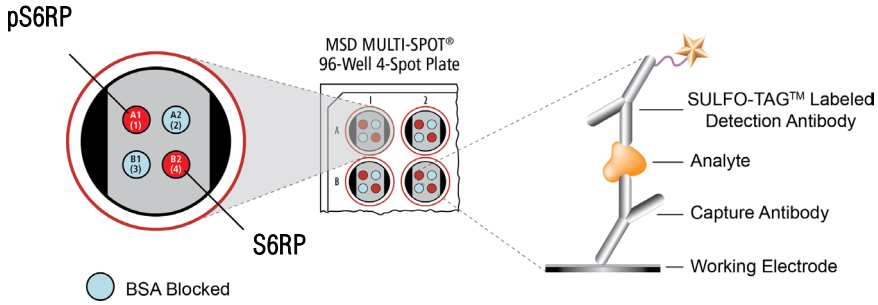

S6 Ribosomal Protein (S6RP) is the S6 subunit of the 40S ribosome and it functions to increase translation of mRNA containing a 5′-terminal oligopyramidine tract (5′-TOP).[1] mRNA with a 5'-TOP generally encode proteins involved in the translational machinery, such as proteins involved in ribosome formation.[2] S6RP functions to control translation of proteins which are constituents of the ribosome, and therefore helps to control overall levels of protein translation. The function of S6RP is phosphorylation dependent, and S6RP is phosphorylated by P70S6K in a mitogen dependent fashion.[3] Residues Ser235, Ser236, Ser240, and Ser244, located within the C-terminus of S6RP, are phosphorylated and important for activation of S6RP.[4]

The MSD Phospho(Ser240/244)/Total S6RP Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Ser240/244)/Total S6RP 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-S6RP and total S6RP antibodies and are shown below for comparison.
Growing low density Jurkat cells were treated with LY294002 (50 µM, 2.5 hours) (negative) or PMA (200 nM, 15 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-S6RP (Ser240/244) and anti-total S6RP antibodies on spatially distinct electrodes within a well. Phosphorylated and total S6RP were detected with anti-total S6RP antibody conjugated with MSD SULFO-TAG™ reagent.

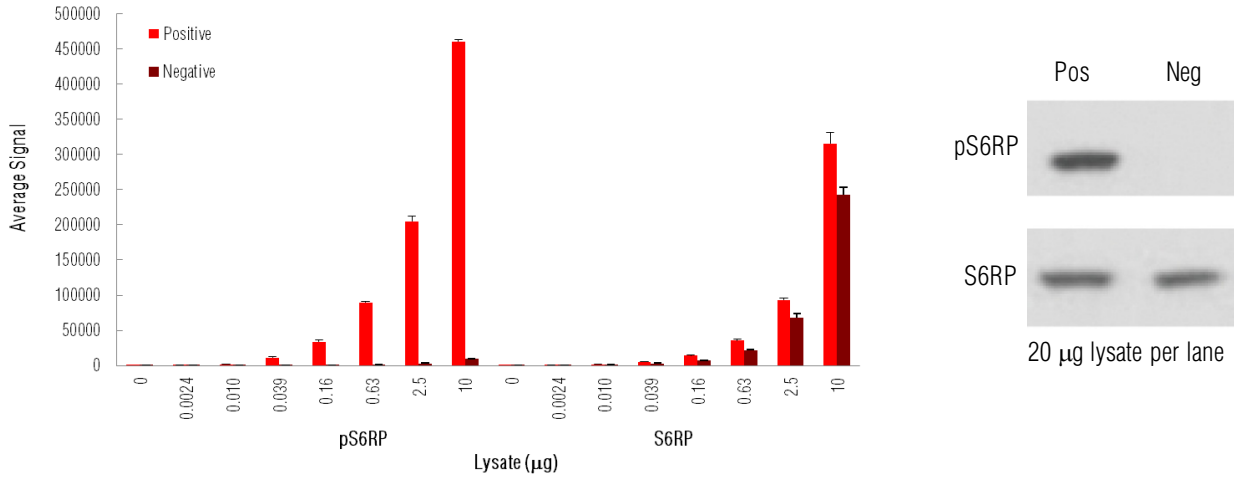

Fig. 1: Sample data generated with the MULTI-SPOT® Phospho(Ser240/244)/Total S6RP Assay. Increased signal for phosphorylated S6RP was observed with only pS6RP positive cell lysate. Total S6RP signal increased throughout the titration of both pS6RP positive and negative cell lysates. The Phospho(Ser240/244)/Total S6RP Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pS6RP positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pS6RP	0	67	19	28.8	67	19	28.8	
	0.0024	582	48	8.3	162	19	11.4	3.6
	0.010	1910	103	5.4	245	29	11.8	7.8
	0.039	11119	1180	10.6	334	47	14.0	33
	0.16	33531	2335	7.0	406	46	11.3	83
	0.63	89400	1566	1.8	1025	34	3.3	87
	2.5	204891	7343	3.6	3106	251	8.1	66
	10	460251	2625	0.6	9479	549	5.8	49
S6RP	0	85	21	24.4	85	21	24.4	
	0.0024	442	29	6.6	325	17	5.1	1.4
	0.010	1698	219	12.9	833	124	14.9	2.0
	0.039	5375	244	4.5	2886	305	10.6	1.9
	0.16	14441	494	3.4	7051	699	9.9	2.0
	0.63	35726	2063	5.8	21425	1689	7.9	1.7
	2.5	93210	2457	2.6	68426	4909	7.2	1.4
	10	315442	15384	4.9	242919	10163	4.2	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

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References

1. Avruch J, Belham C, Weng Q, Hara K, Yonezawa K. The p70 S6 kinase integrates nutrient and growth signals to control translational capacity. Prog Mol Subcell Biol. 2001;26:115-54.
2. Avni D, Biberman Y, Meyuhas O. The 5′ terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. Nucleic Acids Res. 1997 Mar 1;25(5):995-1001.
3. Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, Mueller M, Fumagalli S, Kozma SC, Thomas G. S6K1(−/−)/S6K2(−/−) mice exhibit perinatal lethality and rapamycin-sensitive 5′-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. Mol Cell Biol. 2004 Apr;24(8):3112-24.
4. Flotow H, Thomas G. Substrate recognition determinants of the mitogen-activated 70K S6 kinase from rat liver. J Biol Chem. 1992 Feb 15;267(5):3074-8.