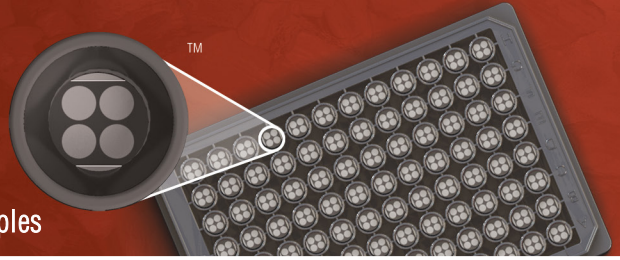
MSD® Phospho-S6RP (Ser235/236) Assay Whole Cell Lysate Kit

For quantitative determination in human, monkey, 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-S6RP (Ser235/236) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150DFD-1
5 plates	K150DFD-2
20 plates	K150DFD-3

Phospho-S6RP Whole Cell Lysate Set	
200 µg	C10DF-1

Ordering information

MSD Customer Service
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Company Address

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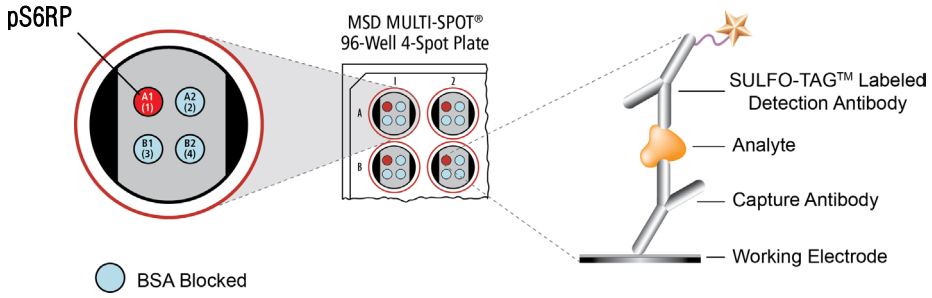

S6 Ribosomal Protein (S6RP) is the S6 subunit of the 40S ribosome and it functions to increase translation of mRNA containing a 5′-terminal oligopyramidine tract (5′-TOP).[1] mRNA with a 5'-TOP generally encode proteins involved in the translational machinery, such as proteins involved in ribosome formation.[2] 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] While mitogens can increase the translation of these 5'-TOP mRNA, rapamycin and analogs of rapamycin can decrease translation of these mRNA leading to the conclusion that S6RP is involved in general control of cell growth.[4] Recent work has brought some of these theories into question as cell lines with mutant versions of S6RP which are incapable of being phosphorylated and cell lines lacking P70S6K are still able to respond to stimuli and increase production of 5'-TOP mRNA.[5,6] Residues Ser235, Ser236, Ser240, and Ser244, located within the C-terminus of S6RP, are phosphorylated and important for activation of S6RP.[7]

The MSD Phospho-S6RP (Ser235/236) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-S6RP (Ser235/236) 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 antibody on one of the four spatially distinct electrodes per well. Phosphorylated S6RP was detected with anti-total S6RP antibody conjugated with MSD SULFO-TAG™ reagent.

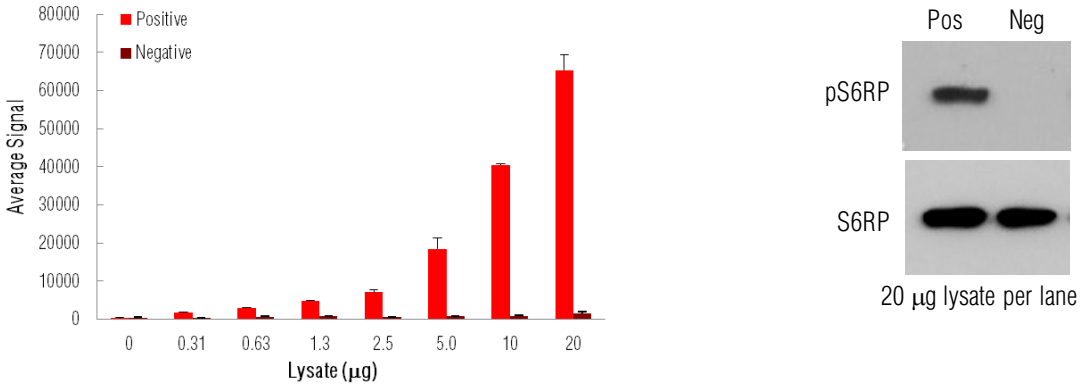

Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-S6RP (Ser235/236) Assay. Increased signal is observed with the titration of pS6RP positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-S6RP (Ser235/236) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pS6RP positive and negative Jurkat cell lysates using the MULTI-ARRAY Phospho-S6RP (Ser235/236) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	333	82	24.6	370	100	27.0	
0.31	1662	110	6.6	409	21	5.1	4.1
0.63	2986	128	4.3	607	256	42.2	4.9
1.3	4840	105	2.2	723	68	9.4	6.7
2.5	7073	585	8.3	628	53	8.4	11
5.0	18467	2762	15.0	744	116	15.6	25
10	40484	281	0.7	910	188	20.7	44
20	65371	3988	6.1	1604	473	29.5	41

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. Hagner PR, Schneider A, Gartenhaus RB. Targeting the translational machinery as a novel treatment strategy for hematologic malignancies. Blood. 2010 Mar 18;115(11):2127-35.
5. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, Dor Y, Zisman P, Meyuhas O. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. Genes Dev. 2005 Sep 15;19(18):2199-211.
6. Stolovich M, Tang H, Hornstein E, Levy G, Cohen R, Bae SS, Birnbaum MJ, Meyuhas O. Transduction of growth or mitogenic signals into translational activation of TOP mRNAs is fully reliant on the phosphatidylinositol 3-kinase-mediated pathway but requires neither S6K1 nor rpS6 phosphorylation. Mol Cell Biol. 2002 Dec;22(23):8101-13.
7. 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.