

# MSD® Phospho-S6RP (Ser240/244) 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-S6RP (Ser240/244) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150DGD-1
5 plates	K150DGD-2
20 plates	K150DGD-3

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

## Ordering information

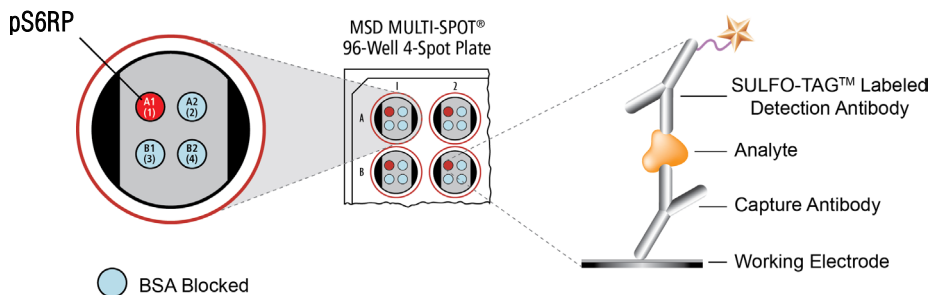
MSD Customer Service  
Phone: 1-301-947-2085  
Fax: 1-301-990-2776  
Email: CustomerService@mesoscale.com

## Company Address

MESO SCALE DISCOVERY®  
A division of  
Meso Scale Diagnostics, LLC.  
9238 Gaither Road  
Gaithersburg, MD 20877 USA

www.mesoscale.com®

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procedures.



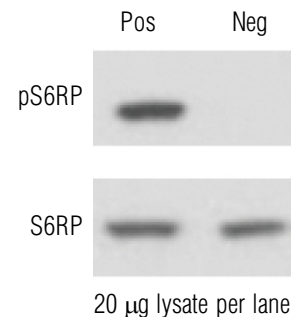
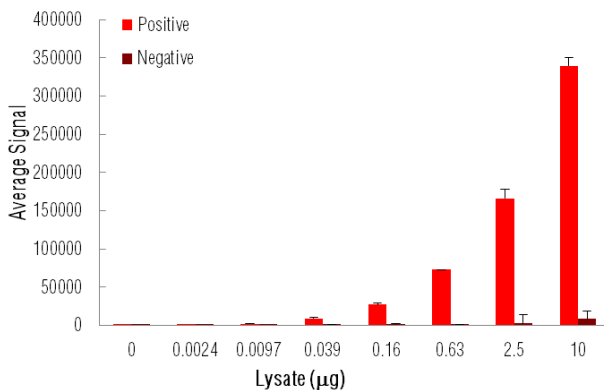
**S6 Ribosomal Protein (S6RP)** is the S6 subunit of the 40S ribosome and it functions to increase translation of mRNA containing a 5'-terminal oligopyrimidine tract (5'-TOP).<sup>1</sup> mRNA with a 5'-TOP generally encode proteins involved in the translational machinery, such as proteins involved in ribosome formation.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup> 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.<sup>5,6</sup> Residues Ser235, Ser236, Ser240, and Ser244, located within the C-terminus of S6RP, are phosphorylated and important for activation of S6RP.<sup>7</sup>

The MSD Phospho-S6RP (Ser240/244) 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 (Ser240/244) 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 (Ser240/244) 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 (Ser240/244) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

# MSD Phosphoprotein Assays

## Lysate Titration

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

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	51	10	18.9	51	10	18.9	
0.0024	494	18	3.7	140	10	7.4	3.5
0.0097	1790	106	5.9	157	11	6.7	11
0.039	9267	860	9.3	225	20	9.0	41
0.16	27391	1378	5.0	393	29	7.4	70
0.63	72717	326	0.4	913	78	8.6	80
2.5	166209	11314	6.8	2766	103	3.7	60
10	339428	10806	3.2	8423	569	6.8	40

## 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](http://www.mesoscale.com)

## References

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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.

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