MSD[®] Phospho-HSP27 (Ser15) Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples



Alzheimer's Disease **BioProcess** Cardiac **Cell Signaling**

Clinical Immunology Cytokines Hypoxia Immunogenicity Inflammation Metabolic Oncology Toxicology Vascular

pHSP27 MSD MULTI-SPOT® 96-Well 4-Spot Plate SULFO-TAG[™] Labeled **Detection Antibody** Analyte Capture Antibody Working Electrode BSA Blocked

HSP27 (Heat Shock Protein 27) is one of the smaller members of the ubiquitous heat shock protein family whose expression is regulated by cellular stresses, growth factors, and inflammatory cytokines. The function of heat shock protein overexpression is to increase cellular resistance to temperature and oxidative shock, chemicals, and other environmental insults. In addition to changes in expression, HSP27 is phosphorylated on several serines (15, 78, 82) during the stress response. HSP27 is phosphorylated by MAPKAP kinase 2 during induction of the p38 MAP kinase pathway. Following phosphorylation, HSP27 undergoes oligometric reorganization to facilitate its molecular chaperone, protein scaffolding, and cellular protective functions. HSP27 also functions to inhibit translation during heat shock by binding to initiation factor eIF4G. Due to the diversity of its protein interactions, HSP27 has been implicated in the control of cell growth, prevention of apoptosis, and smooth muscle cell migration and dysfunction.

The MSD Phospho-HSP27 (Ser15) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-HSP27 (Ser15) 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-HSP27 (Ser15) and total HSP27 antibodies and are shown below for comparison.

Confluent HeLa cell monolayers (negative) were treated with sorbitol (0.4 M; 30 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-HSP27 (Ser15) antibody on one of the four spatially distinct electrodes within a well. Phosphorylated HSP27 was detected with anti-total HSP27 antibody conjugated with MSD SULFO-TAG™ reagent.



Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-HSP27 (Ser15) Assay. Increased signal for phosphorylated HSP27 was observed with pHSP27 positive cell lysate. The Phospho-HSP27 (Ser15) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

pot the Difference

Catalog Numbers

Phospho-HSP27 (Ser15) Assay: Whole Cell Lysate Kit						
Kit size						
1 plate	K150CSD-1					
5 plates	K150CSD-2					
20 plates	K150CSD-3					

Phospho-HSP27 Whole Cell Lysate Set					
200 μ g	C11CS-1				

Ordering information

Company Address

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Lysate Titration

Data for pHSP27 positive and negative HeLa cell lysates using the MULTI-ARRAY Phospho-HSP27 (Ser15) Assay are presented below.

Lysate	Positive			Negative			D/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	P/N
0	51	9	18.2	60	1	2.4	
0.16	769	51	6.6	88	10	11.6	8.7
0.31	2223	241	10.8	164	23	14.1	14
0.63	5589	617	11.0	241	30	12.4	23
1.3	13188	1664	12.6	361	10	2.7	36
2.5	27108	2577	9.5	497	37	7.5	55
5.0	50993	4184	8.2	1033	66	6.3	49
10	89069	12707	14.3	1595	102	6.4	56
20	120198	4612	3.8	2832	258	9.1	42

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 technology for the measurement of phosphoproteins

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- 2. Hendriks BS, Hua F, Chabot JR. Analysis of mechanistic pathway models in drug discovery: p38 pathway. Biotechnol Prog. 2008 Jan-Feb;24(1):96-109. Epub 2007 Oct 5.
- 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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