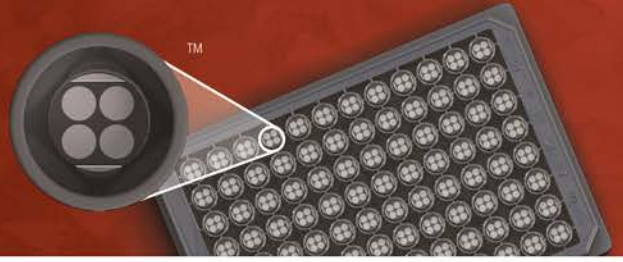
MSD® Phospho(Ser9)/Total GSK-3β Assay Whole Cell Lysate Kit

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 (Ser9)/Total GSK-3β: Whole Cell Lysate Kit	
Kit size	
1 plate	K15109D-1
5 plates	K15109D-2
20 plates	K15109D-3

Phospho-GSK-3β Whole Cell Lysate Set	
200 µg	C11CQ-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
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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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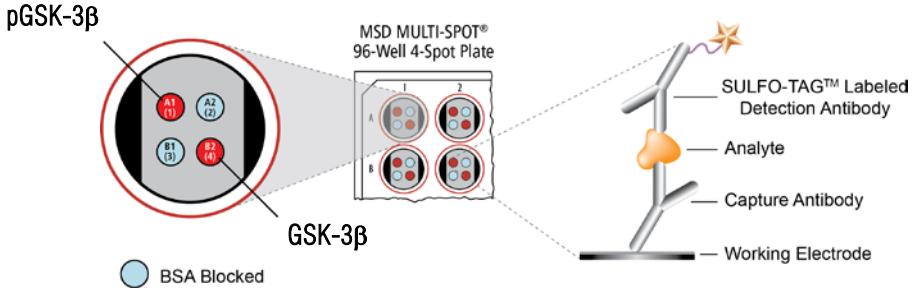
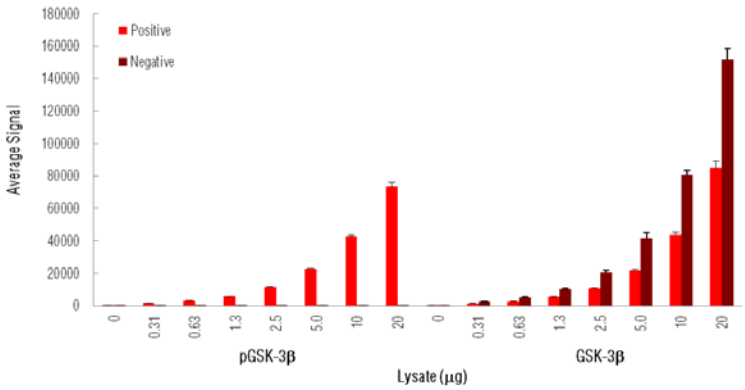


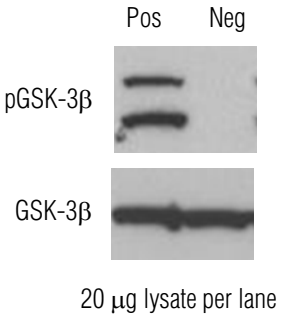
Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that is found in two cellular isoforms –α and -β. GSK-3 has diverse cellular effects including involvement in metabolism, embryonic development, and cell survival. The two isoforms are regulated through phosphorylation, with inhibition as a result of growth factor and insulin-mediated phosphorylation by Akt on Ser 21 (GSK-3α) and Ser 9 (GSK-3β). The inhibition of GSK-3α/GSK-3β results in the dephosphorylation and activation of substrates such as glycogen synthase, eIF-2B, and C/EBPα causing increased protein and glycogen synthesis. Tyrosine (216) phosphorylation of GSK-3β results in its activation and the subsequent phosphorylation of various cellular proteins including cyclin D-1 and β-catenin. An important member of the Wnt signaling pathway, GSK-3 plays a role in cell fate in early embryonic development. GSK-3β has also been implicated in the progression of Alzheimer's disease through the phosphorylation of the microtubule-associated protein tau.

The MSD Phospho(Ser9)/Total GSK-3β Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Ser9)/Total GSK-3β 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-GSK-3β (Ser9) and total GSK-3β antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM; 2.5 hours) and staurosporine (1 µM; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-GSK-3β and anti-total GSK-3β antibodies on spatially distinct electrodes within a well. Phosphorylated and total GSK-3β were detected with anti-total GSK-3β antibody conjugated with MSD SULFO-TAG™ reagent.





20 µg lysate per lane

Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Ser9)/Total GSK-3β Assay. Increased signal for phosphorylated GSK-3β was observed with only pGSK-3β positive cell lysate. Total GSK-3β signal increased throughout the titration of both pGSK-3β positive and negative cell lysates. The Phospho(Ser9)/Total GSK-3β Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pGSK-3β positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Ser9)/Total GSK-3β Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pGSK-3β	0	84	1	1.5	83	6	7.3	
	0.31	1714	19	1.1	138	8	5.7	12
	0.63	3229	18	0.5	139	2	1.4	23
	1.3	5906	74	1.3	153	8	5.1	39
	2.5	11616	233	2.0	178	9	4.9	65
	5.0	22833	339	1.5	192	7	3.5	119
	10	42838	756	1.8	211	6	2.8	203
	20	73605	2307	3.1	264	12	4.7	279
GSK-3β	0	86	5	5.7	80	4	5.4	
	0.31	1428	38	2.7	2621	37	1.4	0.5
	0.63	2776	63	2.3	5283	190	3.6	0.5
	1.3	5464	218	4.0	10552	307	2.9	0.5
	2.5	10730	380	3.5	20767	1034	5.0	0.5
	5.0	21982	361	1.6	41356	3529	8.5	0.5
	10	43866	1290	2.9	80697	2670	3.3	0.5
	20	85136	4068	4.8	152049	6205	4.1	0.6

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. Grimshaw KM, Hunter LJ, Yap TA, Heaton SP, Walton MI, Woodhead SJ, Fazal L, Reule M, Davies TG, Seavers LC, Lock V, Lyons JF, Thompson NT, Workman P, Garrett MD. AT7867 is a potent and oral inhibitor of AKT and p70 S6 kinase that induces pharmacodynamic changes and inhibits human tumor xenograft growth. Mol Cancer Ther. 2010 May;9(5):1100-10.
3. 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. CDK4 and CDK6. Mol Cell Biol. 1995 May;15(5):2672-81..