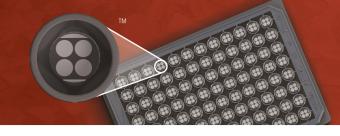
MSD[®] Phospho-GSK-3 α (Ser21) Assay Whole Cell Lysate Kit

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

Catalog Numbers

| Phospho-GSK-3α (Ser21) Assay: Whole Cell Lysate Kit | | | | | | | |
|--|--|--|--|--|--|--|--|
| Kit size | | | | | | | |
| K151C0D-1 | | | | | | | |
| K151C0D-2 | | | | | | | |
| K151C0D-3 | | | | | | | |
| | | | | | | | |

| Phospho-GSK-3α Whole Cell Lysate Set | | | | | | |
|---|---------|--|--|--|--|--|
| 200 μ g | C11CO-1 | | | | | |

Ordering information

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

Company Address

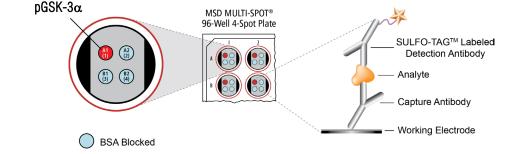
MESO SCALE DISCOVERY® A division of Meso Scale Diagnostics, LLC. 9238 Gaither Road Gaithersburg, MD 20877 USA

www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures. **Fig. 1:** Sample data generated with the MULTI-ARRAY[®] Phospho-GSK-3 α (Ser21) Assay. Increased signal is observed with the titration of pGSK-3 α positive cell lysate. The Phospho-GSK-3 α (Ser21) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

5





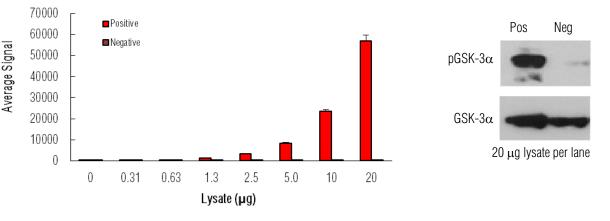
Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase that is found in two cellular isoforms $-\alpha$ and $-\beta$. GSK-3 has diverse cellular effects including involvement in metabolism, embryonic development, and cell survival. The two isoforms are regulated through phosphorylation, with inhibition as a result of growth factor and insulin-mediated phosphorylation by Akt on Ser 21 (GSK-3 α) and Ser 9 (GSK-3 β). The inhibition of GSK-3 α /GSK-3 β results in the dephosphorylation and activation of substrates such as glycogen synthase, eIF-2B, and C/EBP α causing increased protein and glycogen synthesis. Tyrosine (216) phosphorylation of GSK-3 β 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-3 β has also been implicated in the progression of Alzheimer's disease through the phosphorylation of the microtubule-associated protein tau.

The MSD Phospho-GSK-3 α (Ser21) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-GSK- 3α (Ser21) 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- 3α and total GSK- 3α antibodies and are shown below for comparison.

Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 μ M; 2.5 hours) and staurosporine (1 μ M; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT[®] 4-Spot plates coated with anti-total GSK-3 α antibody on one of the four spatially distinct electrodes within a well. Phosphorylated GSK-3 α was detected with anti-phospho-GSK-3 α (Ser21) antibody conjugated with MSD SULFO-TAGTM reagent.



Lysate Titration

Data for pGSK-3 α positive and negative Jurkat cell lysates using the MULTI-ARRAY Phospho-GSK-3 α (Ser21) Assay are presented below.

| Lysate (µg) | Positive | | | Negative | | | D/N |
|----------------|----------------|--------|-----|----------------|--------|------|-----|
| | Average Signal | StdDev | %CV | Average Signal | StdDev | %CV | P/N |
| 0 | 71 | 7 | 9.9 | 60 | 15 | 25.0 | |
| 0.31 | 323 | 16 | 5.0 | 103 | 16 | 15.5 | 3.1 |
| 0.63 | 562 | 26 | 4.6 | 106 | 8 | 7.5 | 5.3 |
| 1.3 | 1253 | 95 | 7.6 | 89 | 13 | 14.6 | 14 |
| 2.5 | 3409 | 73 | 2.1 | 109 | 12 | 11.0 | 31 |
| 5.0 | 8331 | 446 | 5.4 | 93 | 3 | 3.2 | 90 |
| 10 | 23612 | 747 | 3.2 | 106 | 18 | 17.0 | 223 |
| 20 | 56778 | 2822 | 5.0 | 102 | 14 | 13.7 | 557 |

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 <u>www.mesoscale.com</u>

MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, WWW.MESOSCALE.COM, MSD, MSD (DESIGN), DISCOVERY WORKBENCH, QUICKPLEX, MULTI-ARRAY, MULTI-SPOT, SULFO-TAG, SECTOR, SECTOR, SECTOR HTS, SECTOR PR, 4-SPOT (DESIGN) and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC. © 2011 Meso Scale Diagnostics, LLC. All rights reserved.

For Research Use Only. Not for use in diagnostic procedures.

