GSK3 beta pS9 ELISA Kit ab205709

Overview

Product name: GSK3 beta pS9 ELISA Kit
Detection method: Colorimetric

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>6</td>
<td></td>
<td></td>
<td>3%</td>
</tr>
</tbody>
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<tr>
<td>MCF-7</td>
<td>3</td>
<td></td>
<td></td>
<td>6.3%</td>
</tr>
</tbody>
</table>

Sample type: Cell culture extracts, Cell Lysate
Assay type: Semi-quantitative
Sensitivity: 3 µg/ml
Assay time: 1h 30m
Assay duration: One step assay
Species reactivity: Reacts with: Mouse, Human
Predicted to work with: Rat

Product overview:

Abcam’s GSK-3β (pS9) in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit (ab205709) is designed for the semi-quantitative measurement of GSK-3β (pS9) protein in Human and mouse cells.

Glycogen synthase kinase-3 (GSK3) was initially identified as an enzyme that regulated glycogen synthesis in response to insulin, through its ability to phosphorylate and inactivate glycogen synthase. GSK3, expressed as 2 closely related and similarly regulated isoforms, GSK3A and GSK3B. GSK3B is now known to be involved in a diverse array of signaling cellular processes, including glycogen synthesis, cellular adhesion, and it has been implicated in Alzheimer’s disease. GSK3B is an important element of the PI3 kinase/Akt signaling pathway, and its kinase activity is down-regulated by Akt-mediated phosphorylation at Ser9.
An alternative fluorescent substrate, ADHP, can be used with this assay. A microplate reader capable of measuring fluorescence is required if using this product.

**Notes**

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

**Tested applications**

Suitable for: Sandwich ELISA

**Platform**

Pre-coated microplate (12 x 8 well strips)

**Properties**

**Storage instructions**

Store at +4°C. Please refer to protocols.

**Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Wash Buffer PT</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution</td>
<td>1 x 1ml</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR (ab193970)</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>GSK-3β (pS9) Capture Antibody</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>GSK-3β (pS9) Detector Antibody</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>Lyophilized GSK-3β Control Lysate</td>
<td>1 vial</td>
</tr>
<tr>
<td>Plate Seal</td>
<td>1 unit</td>
</tr>
<tr>
<td>SimpleStep Pre-Coated 96 Well Microplate (12 x 8 well strips)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>1 x 12ml</td>
</tr>
</tbody>
</table>

**Function**

Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/beta-catenin. Phosphorylates CTNB1/beta-catenin. Phosphorylates SNAI1. Plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. Prevents the phosphorylation of APC and CLASP2, allowing its association with the cell membrane. In turn, membrane-bound APC allows the localization of MACF1 to the cell membrane, which is required for microtubule capture and stabilization. Phosphorylates MACF1 and this phosphorylation inhibits the binding of
MACF1 to microtubules which is critical for its role in bulge stem cell migration and skin wound repair.

**Tissue specificity**
Expressed in testis, thymus, prostate and ovary and weakly expressed in lung, brain and kidney.

**Sequence similarities**
Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. GSK-3 subfamily. Contains 1 protein kinase domain.

**Post-translational modifications**
Phosphorylated by AKT1 and ILK1. Activated by phosphorylation at Tyr-216.

**Cellular localization**

### Applications

Our Abpromise guarantee covers the use of ab205709 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

### Images

**ELISA Protocol Summary**

Cell line analysis for GSK-3β from 100 µg/mL preparations of cell extracts. Data from triplicate measurements (mean +/- SD) are plotted and compared to 1X Cell Extraction Buffer PTR (zero).
Inhibition of GSK-3β (pS9) phosphorylation in MCF-7 cells in response to UCN-01 treatment. MCF-7 cells were cultured in 96-well tissue culture plates and treated (2 hours) with a dose-range of UCN-01. Cells were then stimulated (15 minutes) with 2.5 µg/mL insulin and lysed. Data from quadruplicate measurements of GSK-3β (pS9) are plotted and compared against total GSK-3β protein levels.

Example of a typical GSK-3β (pS9) cell lysate dilution series. Background-subtracted data values (mean +/- SD) are graphed.

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