Recombinant Human GSK-3 beta His-tag

Catalog Number: 11739-KS

DESCRIPTION

Source

Human embryonic kidney cell, HEK293-derived human GSK-3 beta protein

Met	6-His tag	Sumo-tag (mutated, uncleavable)	3c Protease site	Human GSK-3 beta (Ser2-Thr420) Accession # P49841.2

N-terminal Sequence Analysis

Protein identity confirmed by mass spectrometry

Predicted Molecular Mass

60 kDa

SPECIFICATIONS

SDS-PAGE

55-64 kDa, under reducing conditions

Activity

Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate.

The specific activity is >200 pmol/min/µg, as measured under the described conditions.

Endotoxin Level

<0.10 EU per 1 µg of the protein by the LAL method.

Purity

>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation

Supplied as a 0.2 µm filtered solution in MES, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human GSK-3 beta His-tag (rhGSK-3B) (Catalog # 11739-KS)
- GSK3 Substrate, 1 mg/mL stock in 25 mM Tris, pH 7.5
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhGSK-3B to 5 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 0.4 mg/mL GSK3 substrate in Assay Buffer.
3. Combine equal volumes of 5 µg/mL rhGSK-3B and Substrate Mixture. Create a Substrate Control by replacing enzyme with Assay Buffer.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to wells of a white plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL Kinase Detection Reagent (supplied in kit) to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control

**Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per reaction:

- rhGSK-3B: 2.5 µg/mL
- ATP: 100 µM
- GSK3 Substrate: 0.2 mg/mL

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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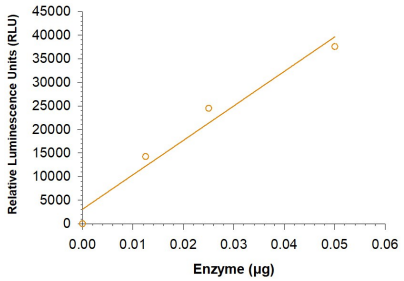
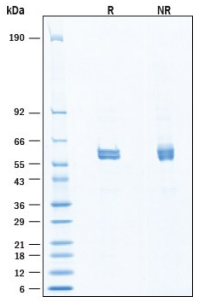
Enzyme Activity



Recombinant Human GSK-3 beta His-tag Enzyme Activity. Recombinant Human GSK-3 beta His-tag (Catalog # 11739-KS) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate.

SDS-PAGE



Recombinant Human GSK-3 beta His-tag SDS-PAGE. 2 µg/lane of Recombinant Human GSK-3 beta His-tag (Catalog # 11739-KS) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 55-64 kDa, under reducing conditions.

BACKGROUND

Glycogen synthase kinase-3 beta (GSK3B) is a ubiquitously expressed, highly conserved serine/threonine kinase (1-3) with key roles in the transduction of regulatory and proliferative signals at the cell membrane. GSK3B is one of two mammalian isoforms of GSK that are constitutively active and have high homology with a kinase domain and an N-terminal phosphorylation site that auto-inhibits its activity by competing with a binding site for preferred pre-phosphorylated substrates (1, 2, 4). The isoforms differ in their sequence similarity at both the N- and C-termini. The GSK3 alpha isoform is primarily cytosolic and has a glycine-rich N-terminal region that excludes it from the nucleus, whereas GSK3B can be found in the nucleus. The different localization differentiates GSK3B biological activity supported by the fact that the GSK3B knockout is embryonically lethal while GSK3 alpha expression is unable to compensate (3). GSK3 has been confirmed to have a diverse and broad range of substrates within multiple cell signaling pathways to regulate glucose metabolism, insulin activity, and energy homeostasis and plays a role in a wide range of cellular processes, such as cell proliferation, differentiation, apoptosis, the cell cycle, the immune response, and organ development (3, 5-6). GSK3B is thus implicated in multiple diseases such as cancer (2, 7) neurodegenerative diseases such as Alzheimer's, bipolar disorder (BPD), psychiatric disorders, Parkinson's, aging (8, 9), inflammatory conditions, and metabolic disorders such as diabetes, cardiovascular disease, and atherosclerosis (2-3, 7-11). There is interest in development of GSK3 inhibitors as a therapeutic target to prevent their ability to phosphorylate their substrates and prevent downstream signaling pathways; clinical trials targeting GSK3 inhibition have been primarily focused on cancers and neurodegenerative and neurological diseases (2-3, 9).

References:

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