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Certificate of Analysis

GSK-3α, active

(Recombinant enzyme expressed in Sf21 insect cells
Item # 14-492, 14-492-K, 14-492M
Parent Lot # D8SN021U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant amino acids 2–end of human GSK-3α expressed in Sf21 insect cells. Purified using Ni2+/NTA-agarose. Purity 59.5% by testing method. MW = 55kDa.

Specific Activity (Parent lot# D8SN021U): 1005U/mg, where one unit of GSK-3α, active activity is defined as 1nmol phosphate incorporated into 200µM GSK-3 substrate peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.692mg/ml of enzyme in 50mM Tris/HCl, pH7.5, 270mM sucrose, 0.1mM EGTA, 150mM NaCl, 1mM benzamidine, 0.03% Brij-35, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

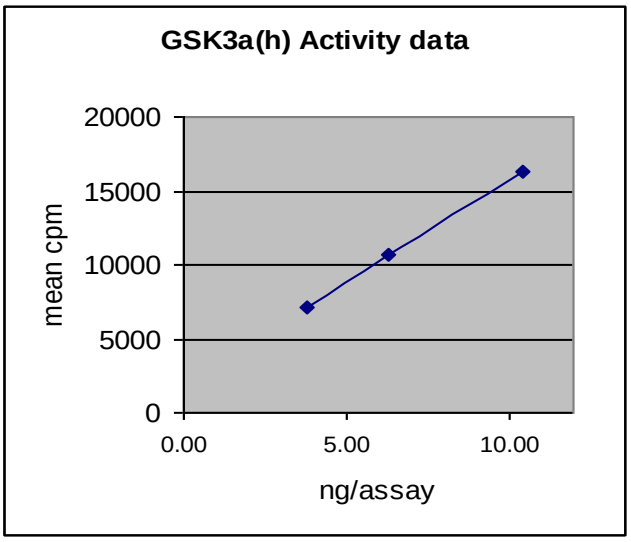
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

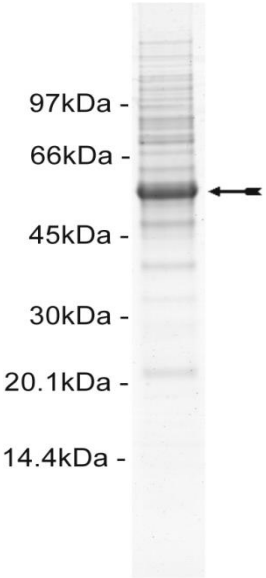
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 3.8–10.4ng of this lot of enzyme phosphorylated 0.5nmol GSK-3 substrate peptide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as GSK3α with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of GSK-3α, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer**: 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **GSK-3 Peptide Substrate (Catalogue# 12-241) (YRRAAVPPSPSLSRHSSPHQS(p)EDEEE):** Use a final assay concentration of 20µM. Make up a 200µM stock. Add 2.5µl of stock per assay point.
3. **GSK-3α, active:** Dilute with 20mM MOPS/NaOH pH7.0, 0.1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 3.8–10.4ng in 2.5µl. per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/ [γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **GSK-3 substrate peptide**.
3. Add 5µl dH_2O.
4. Add **2.5µl (3.8–10.4ng) GSK-3α, active**.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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GSK3α Sequence Information

Protein Human GSK3α

Tags *N*-terminal 6His

Native sequence S10 of the fusion protein is equivalent to S2 of human GSK3α

Accession number GenBank NM_019884. The recombinant GSK3α sequence deviates from that published in NM_019884 by a single amino acid substitution, S449A. This substitution has been reported elsewhere, GenBank 2641993.

Recombinant GSK3α amino acid sequence:

```
  1 MHHHHHHEFS GGGPSGGGPG GSGRARTSSF AEPGGGGGGG GGGPGGSASG PGGTGGGKAS
 61 VGAMGGGVGA SSSGGGPGGS GGGGSGGPGA GTSFPPPGVK LGRDSGKVTT VVATLGQGPE
121 RSQEVAYTDI KVIGNGSFGV VYQARLAETR ELVAIKKVLQ DKRFKNRELQ IMRKLDHCNI
181 VRLRYFFYSS GEKKDELYLN LVLEYVPETV YRVARHFTKA KLTIPILYVK VYMYQLFRSL
241 AYIHSQGVCH RDIKPQNLLV DPDTAVLKLC DFGSAKQLVR GEPNVSYICS RYYRAPELIF
301 GATDYTSSID VWSAGCVLAE LLLGQPIFPG DSGVDQLVEI IKVLGTPTRE QIREMNPNYT
361 EFKFPQIKAH PWTKVFKSRT PPEAIALCSS LLEYTPSSRL SPLEACAHSF FDELRCLGTQ
421 LPNNRPLPPL FNFSAGELSI QPSLNAILIP PHLRSPAGTT TLTPSSQALT ETPTSSDWQS
481 TDATPTLTNS S
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Recombinant GSK3α nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcagc ggcggcgggc cttcgggagg cggccctggg
  61 ggctcgggca gggcgcggac tagctcgttc gcggagcccg gcggcggagg cggaggaggc
 121 ggcggcggcc ccggaggctc ggcctccggc ccaggcggca ccggcggcgg aaaggcatct
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1081 gagttcaagt tccctcagat taaagctcac ccctggacaa aggtgttcaa atctcgaacg
1141 ccgccagagg ccatcgcgct ctgctctagc ctgctggagt acaccccatc ctcaaggctc
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1441 accgatgcca cacctaccct cactaactcc tcctga
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