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Certificate of Analysis

GSK-3β, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-306, 14-306-K, 14-306M
Parent Lot # WAA0024

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 GSK-3β residues 2–end with an H350L mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 92.9% by SDS-PAGE and Coomassie blue staining. MW = 51kDa.

Specific Activity (Parent lot# WAA0024): 808U/mg, where one unit of GSK3β activity is defined as 1nmol phosphate incorporated into 20µM phospho GS2 per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.153mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

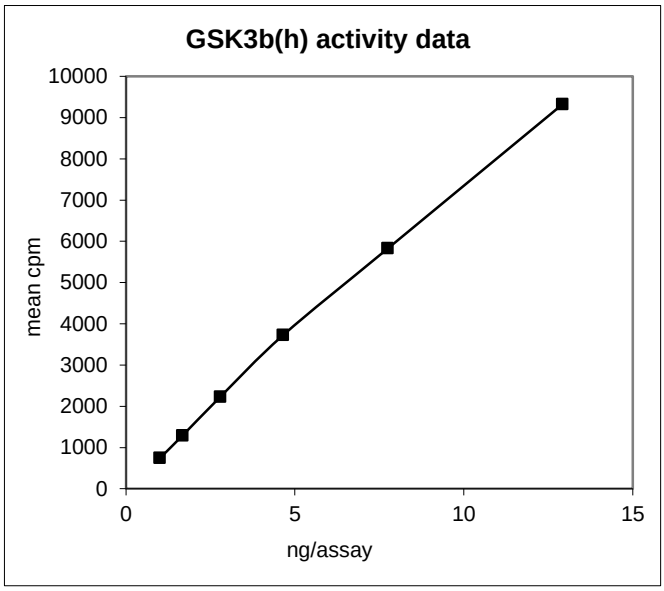
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

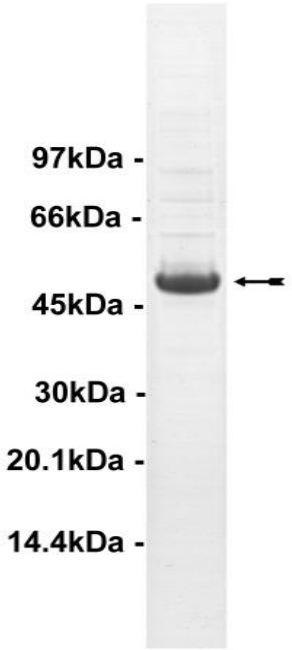
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–13ng of this lot of enzyme phosphorylated 20µM phospho GS2 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 identity as GSK3β 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 active GSK3β.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
2. **Phospho GS2:** Use at a final assay concentration of 20µM. Prepare a 200µm stock. Add 2.5µl of stock per assay point.
3. **GSK3β, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1–13ng 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 well.
2. Add 2.5µl of **phospho GS2**.
3. Add **2.5µl (1–13ng) GSK3β, active**.
4. Add 5µl of dH_2O.
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 50mM 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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GSK3β Sequence Information

Protein human GSK3β (2–end)

Tags *N*-terminal 6His

Native sequence S20 of the recombinant protein is equivalent to S2 of human GSK3β (2–end, H350L)

Accession number EMBL L33801, possessing the amino acid residue substitution H350L. This conflict is reported in the GenBank EST's AI761503 and AL521664, and the March updated version of Swiss Prot P49841.

Recombinant GSK3β amino acid sequence:

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  1 MAHHHHHHGS ATMEFMPMES GRPRTTSFAE SCKPVQQPSA FGSMKVSRDK DGSKVTTVVA
 61 TPGQGPDRPQ EVSYTDTKVI GNGSFGVVYQ AKLCDSGELV AIKKVLQDKR FKNRELQIMR
121 KLDHCNIVRL RYFFYSSGEK KDEVYLNLVL DYVPETVYRV ARHYSRAKQT LPVIYVKLYM
181 YQLFRSLAYI HSFGICHRDI KPQNLLLDPD TAVLKLCDFG SAKQLVRGEP NVSYICSRYY
241 RAPELIFGAT DYTSSIDVWS AGCVLAELLL GQPIFPGDSG VDQLVEIIKV LGTPTREQIR
301 EMNPNYTEFK FPQIKAHPWT KVFRPRTPPE AIALCSRLLE YTPTARLTPL EACAHSFFDE
361 LRDPNVKLPN GRDTPALFNF TTQELSSNPP LATILIPPHA RIQAAASTPT NATAASDANT
421 GDRGQTNNAA SASASNST
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Recombinant GSK3β nucleotide sequence:

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   1 atggcgcatc accatcacca tcacggatct gccaccatgg agttcatgcc catggagtca
  61 gggcggccca gaaccacctc ctttgcggag agctgcaagc cggtgcagca gccttcagct
 121 tttggcagca tgaaagttag cagagacaag gacggcagca aggtgacaac agtggtggca
 181 actcctgggc agggtccaga caggccacaa gaagtcagct atacagacac taaagtgatt
 241 ggaaatggat catttggtgt ggtatatcaa gccaaacttt gtgattcagg agaactggtc
 301 gccatcaaga aagtattgca ggacaagaga tttaagaatc gagagctcca gatcatgaga
 361 aagctagatc actgtaacat agtccgattg cgttatttct tctactccag tggtgagaag
 421 aaagatgagg tctatcttaa tctggtgctg gactatgttc cggaaacagt atacagagtt
 481 gccagacact atagtcgagc caaacagacg ctccctgtga tttatgtcaa gttgtatatg
 541 tatcagctgt tccgaagttt agcctatatc cattcctttg gaatctgcca tcgggatatt
 601 aaaccgcaga acctcttgtt ggatcctgat actgctgtat taaaactctg tgactttgga
 661 agtgcaaagc agctggtccg aggagaaccc aatgtttcgt atatctgttc tcggtactat
 721 agggcaccag agttgatctt tggagccact gattatacct ctagtataga tgtatggtct
 781 gctggctgtg tgttggctga gctgttacta ggacaaccaa tatttccagg ggatagtggt
 841 gtggatcagt tggtagaaat aatcaaggtc ctgggaactc caacaaggga gcaaatcaga
 901 gaaatgaacc caaactacac agaatttaaa ttccctcaaa ttaaggcaca tccttggact
 961 aaggtcttcc gaccccgaac tccaccggag gcaattgcac tgtgtagccg tctgctggag
1021 tatacaccaa ctgcccgact aacaccactg gaagcttgtg cacattcatt ttttgatgaa
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1141 accactcaag aactgtcaag taatccacct ctggctacca tccttattcc tcctcatgct
1201 cggattcaag cagctgcttc aacccccaca aatgccacag cagcgtcaga tgctaatact
1261 ggagaccgtg gacagaccaa taatgctgct tctgcatcag cttccaactc cacctga
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