

Certificate of Analysis

GSK-3 β , 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-3 β residues 2–end with an H350L mutation. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/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 GSK3 β activity is defined as 1nmol phosphate incorporated into 20 μ M phospho GS2 per minute at 30°C with a final ATP concentration of 100 μ M.

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.

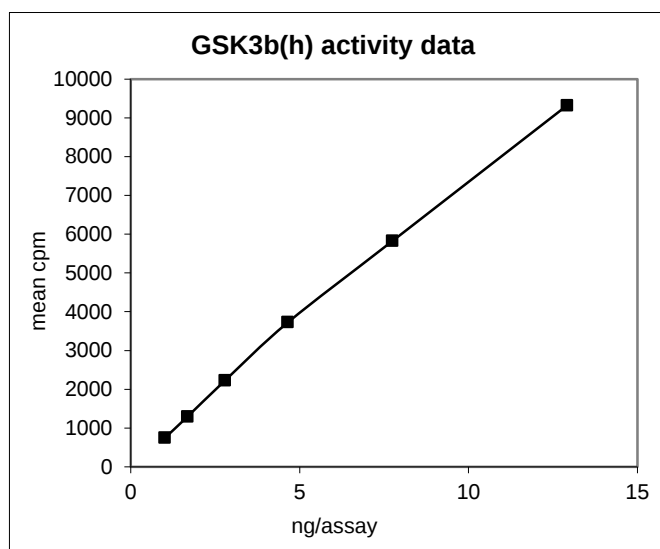
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

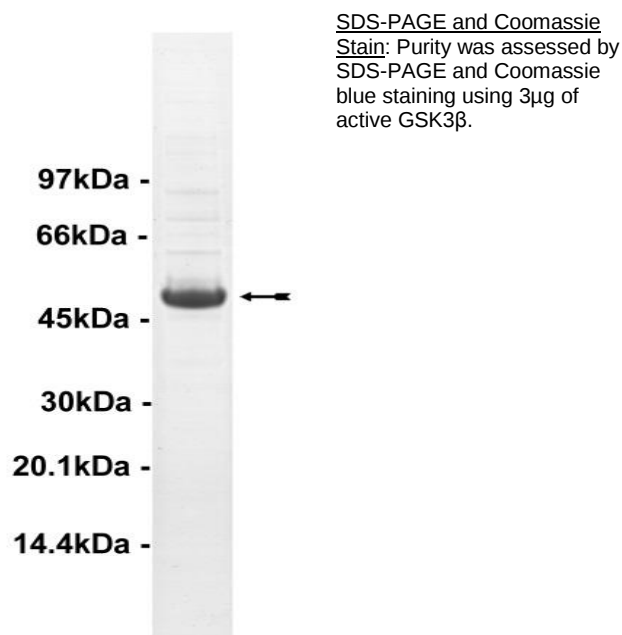
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1–13ng of this lot of enzyme phosphorylated 20 μ M 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 GSK3 β with the translated sequence listed on page three.



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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 20 μ M. Prepare a 200 μ M stock. Add 2.5 μ l of stock per assay point.
3. **GSK3 β , 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 -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per well.
2. Add 2.5 μ l of **phospho GS2**.
3. Add **2.5 μ l (1–13ng) GSK3 β , active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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GSK3 β Sequence Information

<u>Protein</u>	human GSK3 β (2–end)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	S20 of the recombinant protein is equivalent to S2 of human GSK3 β (2–end, H350L)
<u>Accession number</u>	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 GSK3 β amino acid sequence:

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1 MAHHHHHHGS ATMEFMPMES GRPRTTSFAE SCKPVQQPSA FGSMKVS RDK DGSKVTTTVA
61 TPGQGPD RPQ EVSYTDTKVI GNGSFGVVYQ AKLCDSGELV AIKKVLQDKR FKNRELQIMR
121 KLDHCNIVRL RYFFYSSGEK KDEVYLNVLV DYVPETVYRV ARHYSRAKQT LPVIYVKLYM
181 YQLFRSLAYI HSGFICHRDI KPQNLLLDPD TAVLKLCD FG SAKQLVRGEP NVSYICSRY Y
241 RAPELIFGAT DYTSSIDVWS AGCVLAELLL GQPIFPGDSG VDQLVEIIKV LGTPTREQIR
301 EMNPNYTEFK FPQIKAHPWT KVFPRPTPPE AIALCSRLLE YTPTARLTPL EACAHSFFDE
361 LRDPNVKLPN GRDTPALFNF TTQELSSNPP LATILIPPHA RIQAAASTPT NATAASDANT
421 GDRGQTNNAA SASASNST

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Recombinant GSK3 β nucleotide sequence:

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1 atggcgc atc accatcac ca tcacggat ct gccaccat gg agttcatg cc catggagt ca
61 gggcg gcccc aaccac ct ctttgc ggag agctgca agc cgggtgc agca gccttc agct
121 tttggc agca tgaaag tt tag cagagaca ag gacggc agca aggtgaca ac agtggt ggca
181 actcct gggc aggttc caga caggcc acaa gaagtc agct atacagac ac taaagt gatt
241 ggaaat ggat catttg gtgt ggtatat caa gccaaact tt gtgattc agg agaact ggct
301 gccatca aga aagtatt gca ggacaag aga tttaaga atc gagagctcca gatcat gaga
361 aagctag atc actgta acat agtccg attg cgttattt ct tctactcc ag tggtgaga ag
421 aaagat gagg tctatct taa tctggt gctg gactatg ttc cggaaac agt atacagag tt
481 gccagac act atagtc gagc caaacag acg ctccct gtga tttatgt caa gttgtat atg
541 tatcag ctgt tccgaag ttt agcctat atc cattcct ttg gaatctgcca tcgggat att
601 aaaccgc aga acctctt gtt ggatcct gat actgct gtat taaaact ctg tgacttt ggga
661 agtgcaa agc agctgg tccg aggaga accc aatgtttc gt atatctg ttc tcggtact at
721 agggcacc ag agttgat ctt tggagcc act gattata cct ctagtata ga tgtatgg tct
781 gctggct gtg tgttgg ctga gctgtt acta ggacaacca a tatttc cagg ggatagt ggt
841 gtggatc agt tggtagaa at aatcaagg tc ctggga actc caacaagg ga gcaaatc aga
901 gaaatga acc caaacta cac agaatt taaa ttccct caaa ttaagg caca tccttg gact
961 aaggtct ttc gacccc gaac tccacc ggag gcaatt gcac tgtgtag ccg tctgct ggag
1021 tatacaca aa ctgccg act aaccac actg gaagctt gtg cacattc att ttttgat gaa
1081 ttacggg acc caaatgt caa actaccaaat gggcgagaca cacctgc act cttcaact tc
1141 accactca ag aactgt caag taatccac ct ctggctacca tccttatt cc tcctcatg ct
1201 cggattca ag cagctg cttc aaccccc aca aatgccac ag cagcgtc aga tgctaata ct
1261 ggagacc gtg gacagac caa taatgct gct tctgcatc ag cttccaact c cacctga

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