Certificate of Analysis

ProQinase TARGETING CANCER

Recombinant Proteins

GSK3-beta

glycogen synthase kinase 3 beta

Recombinant Human Active Protein Kinase

HGNC Symbol: GSK3B

Synonyms: n/a

Product No.: 0310-0000-1

Lot: 003

Description: Human GSK3-beta, full-length, amino acids M_1-T_{420} (as in NCBI/Protein entry NP_001139628.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: GSK3-beta Lot 003, was confirmed as GSK3-beta by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 76,312 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

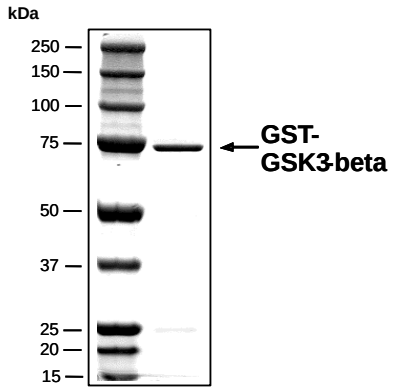
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.276 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

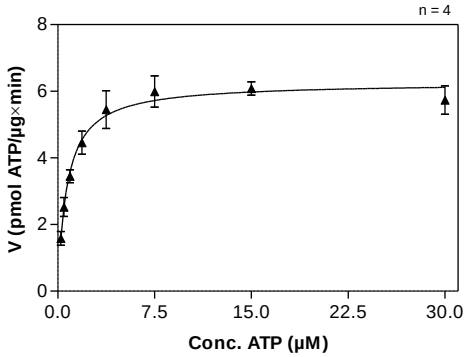
Biochemical Parameters:
Specific kinase activity (P_i transfer): 6.3 pmol/µg × min
ATP-K_M: 0.7 µM

GSK3-beta Lot 003:
Coomassie stain



2.0 µg GST-GSK3-beta

GSK3-beta Lot 003:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml $PEG_{20.000}$
ATP (variable)
Substrate: RBER-IRStide 100 µg/ml
Kinase: 2 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

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GST-GSK3-beta Recombinant Fusion Protein Amino Acid Sequence

```
  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241  LVPRGSPGLD GIYAREMSGR PRTTSFAESC KPVQQPSAFG SMKVSRDKDG SKVTTVVATP 300
301  GQGPDRPQEV SYTDTKVIGN GSFGVVYQAK LCDSGELVAI KKVLQDKRFK NRELQIMRKL 360
361  DHCNIVRLRY FFYSSGEKKD EVYLNLVLDY VPETVYRVAR HYSRAKQTLP VIYVKLYMYQ 420
421  LFRSLAYIHS FGICHRDIKP QNLLLDPDTA VLKLCDFGSA KQLVRGEPNV SYICSRYYRA 480
481  PELIFGATDY TSSIDVWSAG CVLAELLLGQ PIFPGDSGVD QLVEIIKVLG TPTREQIREM 540
541  NPNYTEFKFP QIKAHPWTKV FRPRTPPEAI ALCSRLLEYT PTARLTPLEA CAHSFFDELR 600
601  DPNVKLPNGR DTPALFNFTT QELSSNPPLA TILIPPHARI QAAASTPTNA TAASDANTGD 660
661  RGQTNNAASA SASNST                                                 720
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: GSK3-beta

GSK3-beta wt[1] Amino Acid Sequence

```
1    MSGRPRTTSF AESCKPVQQP SAFGSMKVSR DKDGSKVTTV VATPGQGPDR PQEVSYTDTK  60
61   VIGNGSFGVV YQAKLCDSGE LVAIKKVLQD KRFKNRELQI MRKLDHCNIV RLRYFFYSSG 120
121  EKKDEVYLNL VLDYVPETVY RVARHYSRAK QTLPVIYVKL YMYQLFRSLA YIHSFGICHR 180
181  DIKPQNLLLD PDTAVLKLCD FGSAKQLVRG EPNVSYICSR YYRAPELIFG ATDYTSSIDV 240
241  WSAGCVLAEL LLGQPIFPGD SGVDQLVEII KVLGTPTREQ IREMNPNYTE FKFPQIKAHP 300
301  WTKVFRPRTP PEAIALCSRL LEYTPTARLT PLEACAHSFF DELRDPNVKL PNGRDTPALF 360
361  NFTTQELSSN PPLATILIPP HARIQAAAST PTNATAASDA NTGDRGQTNN AASASASNST 420
```

blue: GSK3-beta sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001139628.1

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