

Certificate of Analysis

TTBK2, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 15-019, 15-019-K, 15-019M
Parent Lot # 192795

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 GST-tagged, recombinant, human TTBK2 amino acids 1-331 expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by size exclusion chromatography. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 65 kDa.

Specific Activity (Parent lot# 192795): 1170 U/mg, where one unit of TTBK2 activity is defined as 1 nmol phosphate incorporated into 2 mg/ml Casein per minute at 30°C with a final ATP concentration of 100 µM.

Formulation: 0.12 mg/ml of enzyme in 50 mM Tris/HCl pH7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

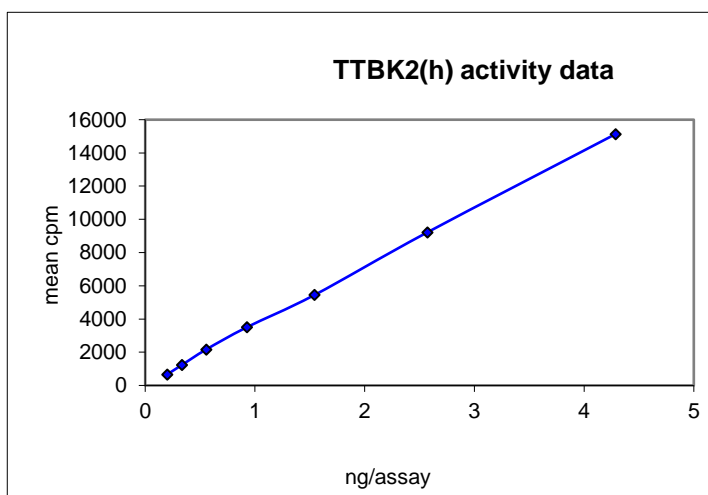
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

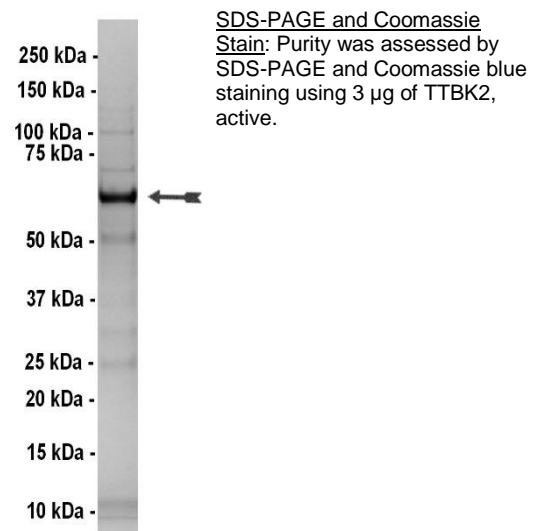
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.20–4.3 ng of this lot of enzyme phosphorylated 2 mg/ml Casein 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 TTBK2 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 5 x Reaction Buffer:** 40 mM MOPS/NaOH pH7.0, 1 mM EDTA.
- Casein:** Use at a final assay concentration of 2 mg/ml. Prepare a 20 mg/ml stock and add 2.5 μ l of stock per assay point.
- TTBK2, active:** Dilute with 20 mM MOPS/NaOH pH7.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 0.20–4.3 ng per assay point.
- $[\gamma\text{-}^{33}\text{P}]\text{ATP}$:** 2.5 x MgAc/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ cocktail: 25 mM MgAc and 0.25 mM ATP to which is added $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

- Add 5.0 μ l of 5 x reaction buffer per assay to wells.
- Add 2.5 μ l of Casein
- Add **2.5 μ l (0.20–4.3 ng) TTBK2, active.**
- Add 5.0 μ l of dH₂O.
- Add 10 μ l of diluted $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5 μ l of 3% phosphoric acid.
- Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
- Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
- 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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TTBK2, active Sequence Information

Protein	Human TTBK2
Tags	N-terminal GST
Native sequence	M236 of the recombinant protein is equivalent to M1 of human TTBK2
Accession number	GenBank BC071556.1

Recombinant TTBK2 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFK GLRRQMSGGG
241 EQPDILSVGI LVKERWKVLR KIGGGGFGEI YDALDMLTRE NVALKVESAQ QPKQVLKMEV
301 AVLKKLQGKD HVCRFIGCGR NDRFNYVVMQ LQGRNLADLR RSQSRGTFTI STTLRLGRQI
361 LESIESIHSV GFLHRDIKPS NFAMGRFPST CRKCYMLDFG LARQFTNSCG DVRPPRAVAG
421 FRGTVRYASI NAHRNREMGR HDDLWSLFYM LVEFVVGQLP WRKIKDKEQV GSIKERYDHR
481 LMLKHLPPPEF SIFLDHISSL DYFTKPDYQL LTSVFDNSIK TFGVIESDPF DWEKTGNDGS
541 LTTTTTSTTP QLHTRLTPAA IGIANA

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Recombinant TTBK2 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgaacttctt
61 ttggaatattc ttgaagaaaa atatgaagag catttgatag agcgcgatga aggtgataaaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
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241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
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1621 ctaacaacca ccactacttc taccaccctc cagttgcaca ctcgcttgac ccctgctgca
1681 attggaattg ccaatgctta a

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