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Certificate of Analysis

TTK, active
(Recombinant enzyme expressed in Sf21 insect cells)
Item # 15-014, 15-014-K, 15-014M
Parent Lot # 226345

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 TTK full length, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 64% by SDS-PAGE and Coomassie blue staining. MW = 124 kDa.

Specific Activity (Parent lot# 226345): 54 U/mg, where one unit of TTK activity is defined as 1 nmol phosphate incorporated into 0.33 mg/ml myelin basic protein per minute at 30°C with a final ATP concentration of 100 µM.

Formulation: 0.60 mg/ml of enzyme in 50 mM Tris/HCl pH 8.0, 4 mM glutathione, 266 mM NaCl, 0.09 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 0.9 mM benzamidine, 0.2 mM PMSF, 0.09% 2-mercaptoethanol. Frozen solution.

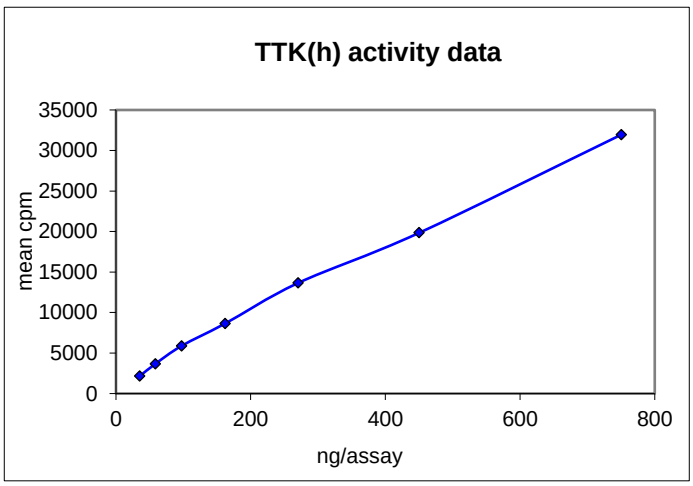
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

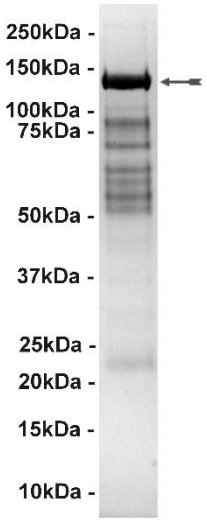
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 35–750 ng of this lot of enzyme phosphorylated 0.33 mg/ml myelin basic protein 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 TTK with the translated sequence listed on page three.



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

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
2. **Myelin basic protein**: Use at a final assay concentration of 0.33 mg/ml. Prepare a 3.33 mg/ml stock and add 2.5 µl of stock per assay point.
3. **TTK, active:** Dilute with 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01% Brij-3 5, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 35–750 ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25 mM MgAc and 0.25 mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 – 800 cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 µl of 5 x reaction buffer per assay to wells.
2. Add 2.5 µl of MBP.
3. Add **2.5 µl (35–750 ng) TTK, active.**
4. Add 5 µl of dH_2O.
5. Add 10 µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 µl of 3% phosphoric acid.
8. Transfer a 10 µl aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the dried filtermat to a sealable plastic bag and add 4 ml 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 µl of 30% phosphoric acid.

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TTK, active Sequence Information

Protein Human TTK

Tags *N*-terminal GST

Native sequence M233 of the recombinant protein is equivalent to M1 of human TTK

Accession number GenBank NM_003318.3
The recombinant protein contains the amino acid substitution S389A with reference to GenBank NM_003318.3. S389A is reported in GenBank M86699.1. The DNA sequence is partially codon optimized for expression purposes and therefore contains non-translational nucleotide base differences with respect to GenBank NM_003318.3.

Recombinant TTK amino acid sequence:

```
   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFG RRMESEDLSG
 241 RELTIDSIMN KVRDIKNKFK NEDLTDELSL NKISADTTDN SGTVNQIMMM ANNPEDWLSL
 301 LLKLEKNSVP LSDALLNKLI GRYSQAIEAL PPDKYGQNES FARIQVRFAE LKAIQEPDDA
 361 RDYFQMARAN CKKFAFVHIS FAQFELSQGN VKKSKQLLQK AVERGAVPLE MLEIALRNLN
 421 LQKKQLLSEE EKKNLSASTV LTAQESFSGS LGHLQNRNNS CDSRGQTTKA RFLYGENMPP
 481 QDAEIGYRNS LRQTNKTKQS CPFGRVPVNL LNSPDCDVKT DDSVVPCFMK RQTSRSECRD
 541 LVVPGSKPSG NDSCELRNLK SVQNSHFKEP LVSDEKSSEL IITDSITLKN KTESSLLAKL
 601 EETKEYQEPE VPESNQKQWQ AKRKSECINQ NPAASSNHWQ IPELARKVNT EQKHTTFEQP
 661 VFSVSKQSPP ISTSKWFDPK SICKTPSSNT LDDYMSCFRT PVVKNDFPPA CQLSTPYGQP
 721 ACFQQQQHQI LATPLQNLQV LASSSANECI SVKGRIYSIL KQIGSGGSSK VFQVLNEKKQ
 781 IYAIKYVNLE EADNQTLDSY RNEIAYLNKL QQHSDKIIRL YDYEITDQYI YMVMECGNID
 841 LNSWLKKKKS IDPWERKSYW KNMLEAVHTI HQHGIVHSDL KPANFLIVDG MLKLIDFGIA
 901 NQMQPDTTSV VKDSQVGTVN YMPPEAIKDM SSSRENGKSK SKISPKSDVW SLGCILYYMT
 961 YGKTPFQQII NQISKLHAII DPNHEIEFPD IPEKDLQDVL KCCLKRDPKQ RISIPELLAH
1021 PYVQIQTHPV NQMAKGTTEE MKYVLGQLVG LNSPNSILKA AKTLYEHYSG GESHNSSSSK
1081 TFEKKRGKK
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Recombinant TTK nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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 661 ctggttccgc gtggatccaa ggaattcggt cgacgaatgg aatctgaaga tctgtctgga
 721 agagagttga caatcgactc cattatgaac aaggtgaggg atattaagaa caaatttaag
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 841 tccggaactg tgaaccagat tatgatgatg gcaaacaacc ccgaagactg gctgagcttg
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Reviewed and approved by site quality representative.