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Certificate of Analysis

Txk, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-761, 14-761-K, 14-761M
Parent Lot # 1773507

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 human Txk, residues 256–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 66.4% by SDS-PAGE and Coomassie staining. MW = 35.3kDa.

Specific Activity (Parent lot# 1773507): 4149U/mg, where one unit of Txk, active activity is defined as 1nmol phosphate incorporated into 250µM (GEEPLYWSFPAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.358mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

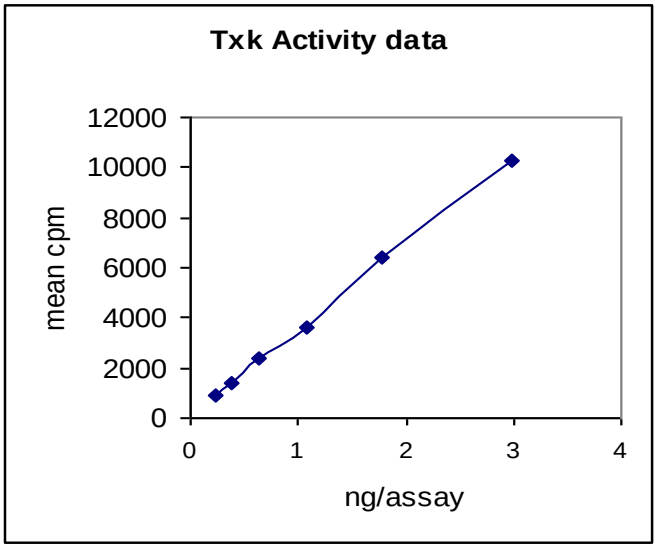
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

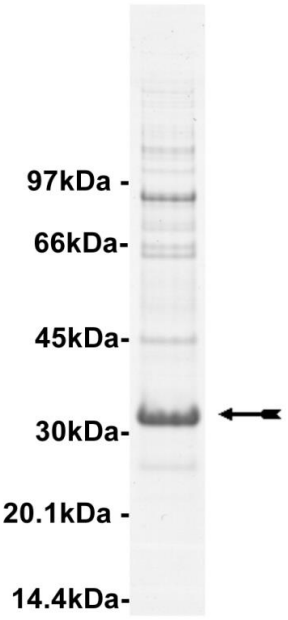
Quality Control Testing

Kinase Assay: 0.2–3.0ng of this lot of enzyme phosphorylated 250µM (GEEPLYWSFPAKKK) 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 Txk 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 Txk, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(GEEPLYWSFPAKKK):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **Txk, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–3.0ng 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 assay to wells.
2. Add 2.5µl of **(GEEPLYWSFPAKKK)**.
3. Add **2.5µl (0.2–3.0ng) Txk, 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 75mM 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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Txk Sequence Information

Protein Human Txk

Tags *N*-terminal 6His

Native sequence T31 of recombinant sequence is equivalent to T256 of native human TXK

Accession number GenBank NM_003328

Recombinant Txk amino acid sequence:

```
  1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF TAGFSYEKWE IDPSELAFIK EIGSGQFGVV
 61 HLGEWRSHIQ VAIKAINEGS MSEEDFIEEA KVMMKLSHSK LVQLYGVCIQ RKPLYIVTEF
121 MENGCLLNYL RENKGKLRKE MLLSVCQDIC EGMEYLERNG YIHRDLAARN CLVSSTCIVK
181 ISDFGMTRYV LDDEYVSSFG AKFPIKWSPP EVFLFNKYSS KSDVWSFGVL MWEVFTEGKM
241 PFENKSNLQV VEAISEGFRL YRPHLAPMSI YEVMYSCWHE KPEGRPTFAE LLRAVTEIAE
301 TW
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Recombinant Txk nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgga tccggaattc acagctgggt ttagctacga aaagtgggag
121 atagatccat ctgagttggc ttttataaag gagattggaa gcggtcagtt tggagtggtc
181 catttaggtg aatggcggtc acatatccag gtagctatca aggccatcaa tgaaggctcc
241 atgtctgaag aggatttcat tgaagaggcc aaagtgatga tgaaattatc tcattcaaag
301 ctagtgcaac tttatggagt ctgtatacag cggaagcccc tttacattgt gacagagttc
361 atggaaaatg gctgcctgct taactatctc agggagaata aaggaaagct taggaaggaa
421 atgctactga gtgtatgcca ggatatatgt gaaggaatgg aatatctgga gaggaatggc
481 tatattcata gggatttggc ggcaaggaat tgtttggtca gctcaacatg catagtaaaa
541 atttcagact ttggaatgac aaggtacgtt ttggatgatg agtatgtcag ttcttttgga
601 gccaagttcc caatcaagtg gtcccctcct gaagtttttc ttttcaataa gtacagcagt
661 aaatctgatg tctggtcatt tggagtttta atgtgggaag tttttacaga aggaaaaatg
721 ccttttgaaa ataagtcaaa tttgcaagtc gtggaagcta tttctgaagg cttcaggcta
781 tatcgccctc acctggcacc aatgtccata tatgaagtca tgtacagctg ctggcatgag
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901 acctggtga
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Reviewed and approved by site quality representative.