

Certificate of Analysis

Tec, activated

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-801, 14-801-K, 14-801M

Parent Lot # D8PN044U

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 Tec amino acids 174–end expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Autoactivated by incubating with Mg/ATP, excess ATP and MgAc removed by gel filtration. Purity 74.6% by SDS-PAGE and Coomassie blue staining. MW = 57kDa.

Specific Activity (Parent lot# D8PN044U): 1400U/mg, where one unit of Tec, activated activity is defined as 1nmol phosphate incorporated into 400μM (EFPIYDFLPAKKK) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.526mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5mM β-glycerophosphate, 1mM Na₃VO₄. Frozen solution.

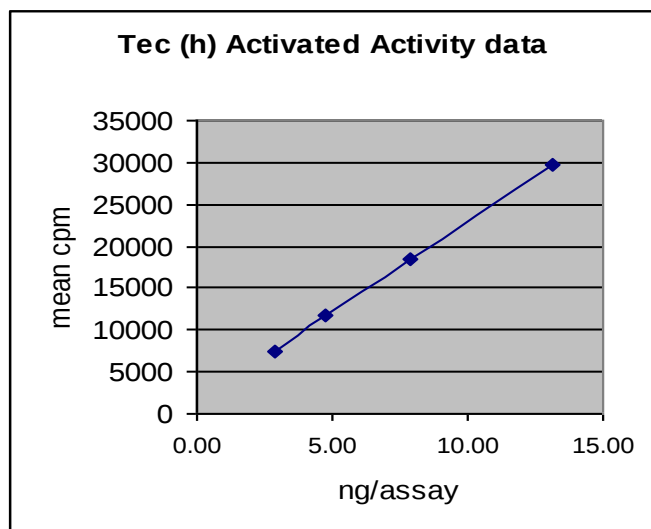
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

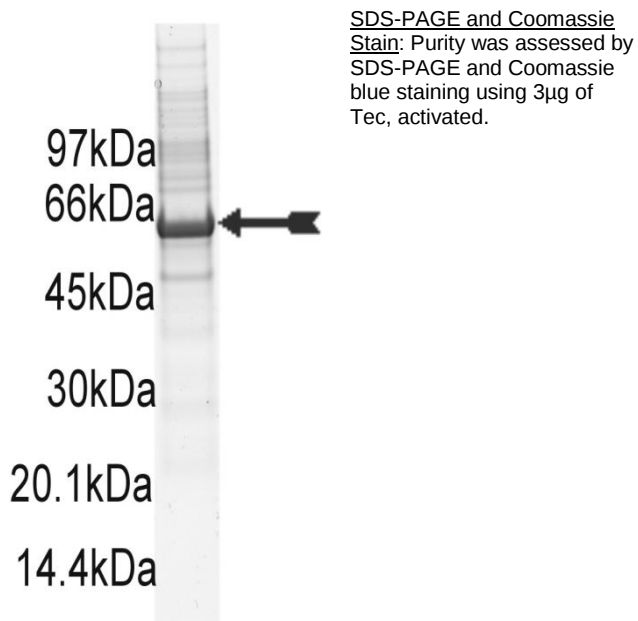
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.9–13.2ng of this lot of enzyme phosphorylated 400μM (EFPIYDFLPAKKK) 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 Tec with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na₃VO₄:** Use at a final assay concentration of 1mM. Prepare a 100mM stock and add 0.25µl of stock per assay point.
3. **Na-β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock and add 0.125µl of stock per assay point.
4. **(EFPIYDFLPAKKK):** Use at a final assay concentration of 400µM. Prepare a 4mM stock and add 2.5µl of stock per assay point.
5. **Tec, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.9–13.2ng per assay point.
6. **[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(EFPIYDFLPAKKK)**.
3. Add **2.5µl (2.9–13.2ng) Tec, activated**.
4. Add 4.625µl of dH₂O.
5. Add 0.25µl Na₃VO₄
6. Add 0.125µl Na-β-glycerophosphate
7. Add 10µl of diluted [γ-³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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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Tec Sequence Information

<u>Protein</u>	Human Tec
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	L31 of the recombinant protein is equivalent to L174 of human Tec
<u>Accession number</u>	GenBank NM_003215. The recombinant protein contains the amino acid substitution F514V with reference to NM_003215. F514V is reported in GenBank CB985022.

Recombinant Tec amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF LEEEDNSEEI VVAMYDFQAA EGHDLRLERG
61 QEYLILEKND VHWWRRARDKY GNEGYIPSNY VTGKKSNNLD QYEWYCRNMN RSKAEQLLRS
121 EDKEGGFMVR DSSQPGLYTV SLYTKFGGEG SSGFRHYHIK ETTTSPKKYY LAEKHAFGSI
181 PEIIEYHKHN AAGLVTRLRY PVSVKGNAP TTAGFSYEKW EINPSELTfM RELGSGLFGV
241 VRLGKWRAQY KVAIKAIREG AMCEEDFIEE AKVMMKLTHP KLVQLYGVCT QQKPIYIVTE
301 FMERGCCLNF LRQRQGHFSR DVLLSMCQDV CEGMEYLERN SFIHRDLAAR NCLVSEAGVV
361 KVSDFGMARY VLDDQYTSSS GAKFPVKWCP PEVFNYSRFS SKSDVWSFGV LMWEVFTEGR
421 MPFEKYTNYE VVTMVTRGHR LYQPKLASNY VYEVMLRCWQ EKPEGRPSFE DLLRTIDELV
481 ECEETFGR

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Recombinant Tec nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatgga tccggaattc ctagaagaag aagataatag tgaagaaatc
121 gttgtagcca tgtatgattt ccaagcagca gaaggacatg atctcagatt agagagaggc
181 caagagtatc tcatttttaga aaagaatgat gttcattggt ggagagcaag agataaatat
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1441 gaatgtgaag aaacttttgg aagataa

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