

Certificate of Analysis

TrkC, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-922, 14-922-K, 14-922M

Parent Lot # D12KP023N

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 and C-terminal 6His-tagged, recombinant, human TrkC amino acids 510-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose followed by gel filtration.

Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 64kDa.

Specific Activity (Parent lot# D12KP023N): 998U/mg, where one unit of TrkC, active activity is defined as 1nmol phosphate incorporated into 500µM (GEEPLYWSFPAKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.308mg/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. Frozen solution.

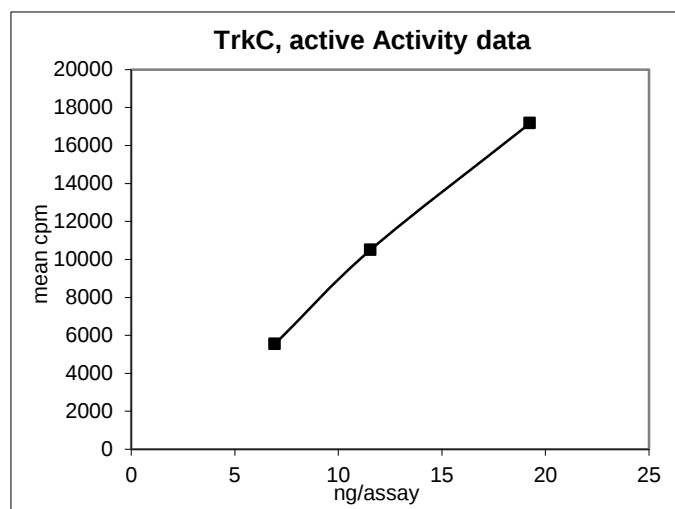
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

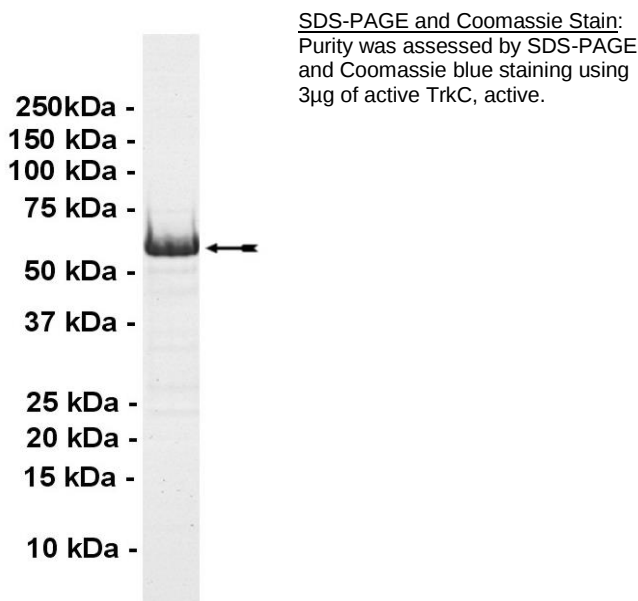
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 7–19ng of this lot of enzyme phosphorylated 500µM of (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 TrkC 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. **(GEEPLYWSFPAKKK):** Use at a final assay concentration of 500 μ M. Prepare a 2.5mM stock and add 5 μ l of stock per assay point.
3. **TrkC, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–19ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 5 μ l of **(GEEPLYWSFPAKKK)**.
3. Add **2.5 μ l (7–19ng) TrkC**.
4. Add 2.5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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TrkC Sequence Information

<u>Protein</u>	human TrkC
<u>Tags</u>	N-terminal GST and C-terminal 6His
<u>Native sequence</u>	V231 of the recombinant protein is equivalent to V510 of human TrkC
<u>Accession number</u>	GenBank NM_002530

Recombinant TrkC amino acid sequence:

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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  LVPRGSKEFM  VIENPQYFRQ
241 GHNCHKPDY  VQHIKRRDIV  LKRELGECAF  GKVFLAECYN  LSPTKDKMLV  AVKALKDPTL
301 AARKDFQREA  ELLTNLQHEH  IVKFYGVCGD  GDPLIMVFEY  MKHGDNLNKL  RAHGPDAMIL
361 VDGQPRQAKG  ELGLSQMLHI  ASQIASGMVY  LASQHFVHRD  LATRNCLVGA  NLLVKIGDFG
421 MSRDVYSTDY  YRVGGHTMLP  IRWMPPESES  YRKFTTESDV  WSFGVILWEI  FTYGKQPWFQ
481 LSNTIEVIECI  TQGRVLERPR  VCPKEVYDVM  LGCWQREPQQ  RLNIKEIYKI  LHALGKATPI
541 YLDILGHHHH  HH

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Recombinant TrkC nucleotide sequence:

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1  atgtccccta  tactagggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatatg  agcgcgatga  aggtgataaa
121 tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatattg
181 ggtgatgtta  aattaacaca  gtctatggcc  atcatacgtt  atatagctga  caagcacaac
241 atgttggttg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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421 acatatatta  atggtgatca  tgtaacccat  cctgacttca  tggtgatga  cgctcttgat
481 gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541 aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601 tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcgcat
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1321 attcgctgga  tgctccttga  aagcatcatg  taccggaagt  tcactacaga  gagtgatgta
1381 tggagcttcg  ggggtgatcc  ctgggagatc  ttcacctatg  gaaagcagcc  atggttccaa
1441 ctctcaaaaca  cggagggtcat  tgagtgcatt  acccaagggtc  gtgttttgga  gcggccccga
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1561 cggttgaaca tcaaggagat ctacaaaatc ctccatgctt tggggaaggc caccccaatc
1621 tacctggaca ttcttgcca ccataccat caccattaa

Reviewed and approved by site quality representative.

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