

## 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.

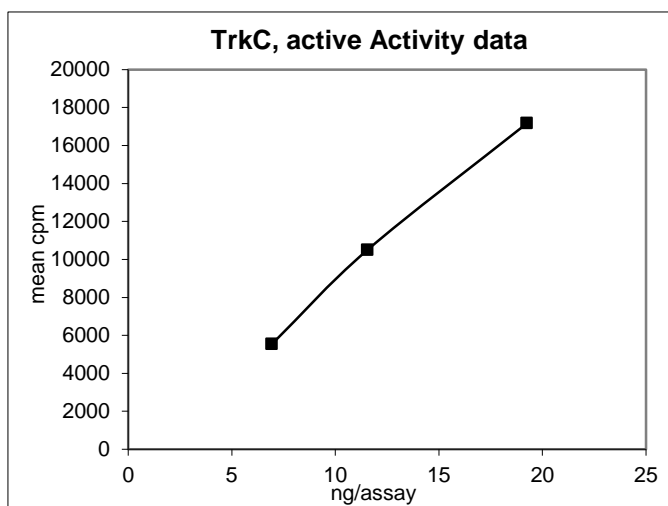
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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 $\mu$ M. Prepare a 2.5mM stock and add 5 $\mu$ 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. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x MgAc/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 5 $\mu$ l of **(GEEPLYWSFPAKKK)**.
3. Add **2.5 $\mu$ l (7–19ng) TrkC**.
4. Add 2.5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ 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 $\mu$ l of 30% phosphoric acid.

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### TrkC Sequence Information

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

#### Recombinant TrkC amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGQATF GGDHPPKSD LVPRGSKEFM VIENPQYFRQ
241 GHNCHKPDTY VQHIKRRDIV LKRELGECAF GKVFLAECYN LSPTKDKMLV AVKALKDPTL
301 AARKDFQREA ELLTNLQHEH IVKFYGVCGD GDPLIMVFEY MKHGDINKFL RAHGPDAMIL
361 VDGQPRQAKG ELGLSQMLHI ASQIASGMVY LASQHFVHRD LATRNCLVGA NLLVKIGDFG
421 MSRDVYSTDY YRVGGHTMLP IRWMPPEM YRKFTTESDV WSFGVILWEI FTYGKQPFQ
481 LSNTEVIECI TQGRVLERPR VCPKEVYDVM LGCWQREPQQ RLNIKEIYKI LHALGKATPI
541 YLDILGHHHH HH

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#### Recombinant TrkC nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acgggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atgggtgatca tgaacccat cctgacttca tgttgatga cgctctgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgtt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatccaa ggaattcatg gtcattgaga acccccagta cttccgctag
721 ggacacaact gccacaagcc ggacacgat gtgcagcaca ttaagaggag agacatcgtg
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1141 gccagtcaga tcgcctcggg tatgggtgtac ctggcctccc agcactttgt gcaccgagac
1201 ctggccacca ggaactgcct ggttggagcg aatctgctag tgaagattgg ggacttcggc
1261 atgtccagag atgtctacag cacggattat tacagggtgg gaggacacac catgctcccc
1321 attcgtgga tgctctctga aagcatcatg taccggaagt tctactacaga gagtgatgta
1381 tggagcttcg gggtgatcct ctgggagatc ttcacctatg gaaagcagcc atggttccaa
1441 ctctcaaaaca cggaggtcat tgagtgcatt acccaaggtc gtgttttggg gcggccccga
1501 gtctgccccca aagaggtgta cgatgtcatg ctgggggtgct ggcagagggga accacagcag

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## Certificate of Analysis

1561 cggttgaaca tcaaggagat ctacaaaatc ctccatgctt tggggaaggc cacccaatc  
1621 tacctggaca ttcttgcca ccatcaccat caccattaa

Reviewed and approved by site quality representative.

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