

## Certificate of Analysis

### TrkB (455–end), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-507, 14-507-K, 14-507M

Parent Lot # WAE0375

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 TrkB, amino acids 455–end, expressed in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 42.9kDa.

**Specific Activity (Parent lot# WAE0375):** 371U/mg, where one unit of TrkB, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM and an enzyme concentration of 171 ng/well.

**Formulation:** 0.76mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

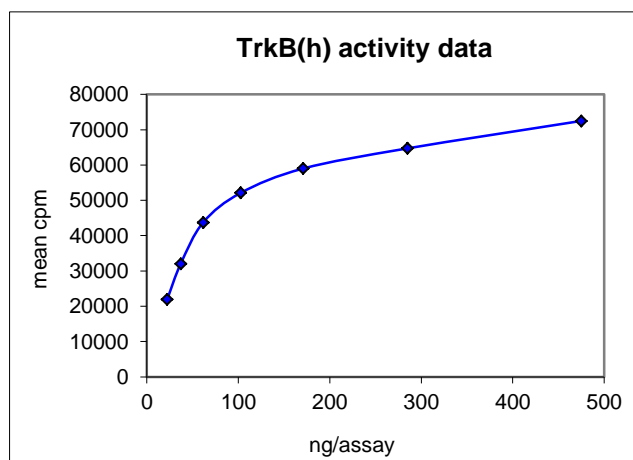
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

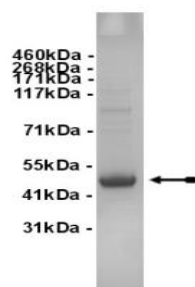
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 22.16–475ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu,Tyr) (4:1) 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 product identity as human TrkB 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 TrkB, active.

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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS, pH7.0, 1mM EDTA.
2. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Add 2.5 µl of stock per assay point.
3. **TrkB, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 22.16–475ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (22.16–475ng) TrkB, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>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 **Filtermat A**.
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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## TrkB Sequence Information

<b><u>Protein</u></b>	Human TrkB
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	K10 of the recombinant protein is equivalent to K455 of human TrkB
<b><u>Accession number</u></b>	GenBank U12140

### Recombinant TrkB amino acid sequence:

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1  MHHHHHHEFK  LARHSKFGMK  GPASVISNDD  DSASPLHHIS  NGSNTPSSSE  GGPDAVIIGM
61  TKIPVIENPQ  YFGITNSQLK  PDTFVQHIKR  HNIVLKRELG  EGAFGKVFLA  ECYNLCPEQD
121 KILVAVKTLK  DASDNARKDF  HREAELLTNL  QHEHIVKFYG  VCVEGDPLIM  VF EYMKHGDL
181 NKFLRAHGP  AVLMAEGNPP  TELTQSQMLH  IAQQIAAGMV  YLASQHFVHR  DLATRNCLVG
241 ENLLVKIGDF  GMSRDVYST  YYRVGGHTML  PIRWMPPEI  MYRKFTTESD  VWSLGVVLWE
301 IFTYGKQPWY  QLSNNEVIE  ITQGRVLQRP  RTCPQEVYEL  MLGCWQREPH  MRKNIKIHT
361 LLQNLAKASP  VYLDILG

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### Recombinant TrkB nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcaag  ttggcaagac  actccaagtt  tggcatgaaa
61  ggcccagcct  ccgttatcag  caatgatgat  gactctgcca  gccactcca  tcacatctcc
121 aatgggagta  aactccatc  ttcttcggaa  ggtggcccag  atgctgtcat  tattggaatg
181 accaagatcc  ctgtcattga  aaatccccag  tactttggca  tcaccaacag  tcagctcaag
241 ccagacacat  ttgttcagca  catcaagcga  cataacattg  ttctgaaaag  ggagctaggc
301 gaaggagcct  ttggaaaagt  gttcctagct  gaatgctata  acctctgtcc  tgagcaggac
361 aagatcttgg  tggcagtga  gaccctgaag  gatgccagt  acaatgcacg  caaggacttc
421 caccgtgagg  ccgagctcct  gaccaacctc  cagcatgagc  acatcgtcaa  gttctatggc
481 gtctgcgtgg  agggcgaccc  cctcatcatg  gtctttgagt  acatgaagca  tggggacctc
541 aacaagttcc  tcagggcaca  cggccctgat  gccgtgctga  tggctgaggg  caaccgccc
601 acggaactga  cgagtcgca  gatgtgcat  atagcccagc  agatcgccgc  gggcatggtc
661 tacctggcgt  cccagcactt  cgtgcaccgc  gatttggcca  ccaggaactg  cctggtcggg
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781 tactacaggg  tcggtggcca  cacaatgctg  cccattcgct  ggatgcctcc  agagagcatc
841 atgtacagga  aattcacgac  ggaaagcgac  gtctggagcc  tgggggtcgt  gttgtgggag
901 attttcacct  atggcaaaca  gccctggtag  cagctgtcaa  acaatgaggt  gatagagtgt
961 atcactcagg  gccgagtcct  gcagcgaccc  cgacgtgcc  cccaggaggt  gtatgagctg
1021 atgctggggt  gctggcagcg  agagcccac  atgaggaaga  acatcaaggg  catccatacc
1081 ctccctcaga  acttgccaa  ggcactctcg  gtctacctgg  acattctagg  ctg

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