

Certificate of Analysis

TRKA, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-571, 14-571-K, 14-571M

Parent Lot # WAA0104

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 TRKA, residues 440–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 67% by SDS-PAGE and Coomassie blue staining. MW = 41kDa.

Specific Activity (Parent lot# WAA0104): 265U/mg, where one unit of TRKA, active activity is defined as 1nmol phosphate incorporated into 250μM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.768mg/ml of enzyme in 50mM Tris/HCl pH8.5 (at 4°C), 300mM NaCl, 5% glycerol, 0.1mM EGTA, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

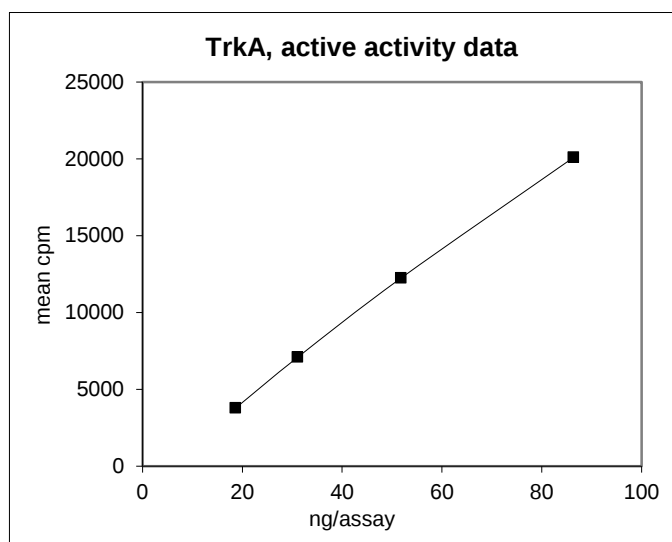
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

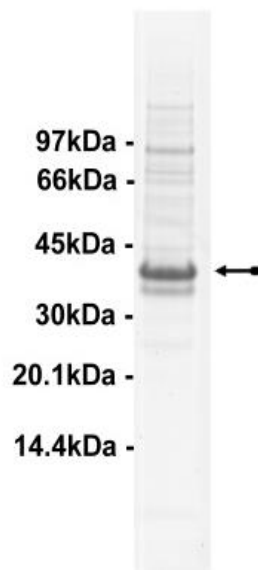
Quality Control Testing

Kinase Assay: 19–86ng of this lot of enzyme phosphorylated 250μM (KKKSPGEYVNIEFG) 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 TRKA 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 TRKA, active.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **TRKA, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 19–86ng per assay point.
4. **[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 to wells.
2. Add 2.5µl of **(KKKSPGEYVNIEFG)**.
3. Add **2.5µl (19–86ng) TRKA, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 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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TRKA Sequence Information

<u>Protein</u>	human TRKA
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	N10 of the recombinant protein is equivalent to N440 of human TRKA
<u>Accession number</u>	GenBank NM_002529

Recombinant TRKA amino acid sequence:

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1  MHHHHHHEFN  KCGRRNKFGI  NRPVLAPEE  GLAMSLHFMT  LGGSSLSPT  GKGSGLQGHI
61  IENPQYFSDA  CVHHIKRDI  VLKWLGEA  FGKVFLAECH  NLLPEQDKM  VAVKALKEAS
121 ESARQDFQRE  AELLTMLQHQ  HIVRFFGVCT  EGRPLLMVFE  YMRHGDNLRF  LRSHGPDACL
181 LAGGEDVAPG  PLGLGQLLAV  ASQVAAGMVY  LAGLHFVHRD  LATRNCLVGQ  GLVVKIGDFG
241 MSRDISTDY  YRVGGRTMLP  IRWMPPELIL  YRKFTTESDV  WSFGVVLWEI  FTYGKQWPYQ
301 LSNTEAIDCI  TQGRELERPR  ACPPEVYAIM  RGCWQREPQQ  RHSIKDVHAR  LQALAQAPPV
361 YLDVLG

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Recombinant TRKA nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcaac  aaatgtggac  ggagaaacaa  gtttgggatc
61  aaccgcccgg  ctgtgctggc  tccagaggat  gggctggcca  tgtccctgca  ttcatgaca
121 ttgggtggca  gctccctgtc  cccaccgag  ggcaaaggct  ctgggctcca  aggcacatc
181 atcgagaacc  cacaatactt  cagtgtatgc  tgtgttcacc  acatcaagcg  ccgggacatc
241 gtgctcaagt  gggagctggg  ggagggcgcc  tttgggaagg  tcttccttgc  tgagtggcac
301 aacctcctgc  ctgagcagga  caagatgctg  gtggctgtca  aggcactgaa  ggaggcgtcc
361 gagagtgttc  ggcaggactt  ccagcgtgag  gctgagctgc  tcaccatgct  gcagcaccag
421 cacatcgtgc  gcttcttcgg  cgtctgcacc  gagggccgcc  ccctgctcat  ggtctttgag
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1081 tacctgatg  tcctgggcta  g

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