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Certificate of Analysis

TRKA, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-571

Lot # 1659421

From bulk lot # 25726U

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 57% by SDS-PAGE and Coomassie blue staining. MW = 41kDa.

Specific Activity (lot# 1659421): 377U/mg, where one unit of TRKA activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 10µg of enzyme in 5.8µl of 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.

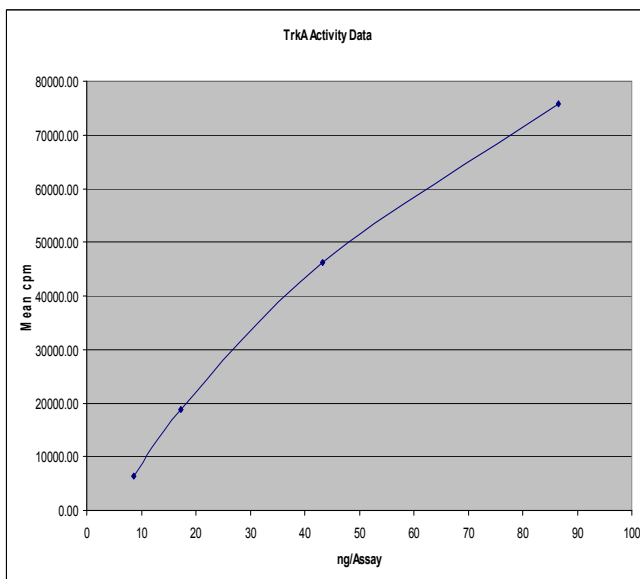
Storage and Stability: Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge 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**

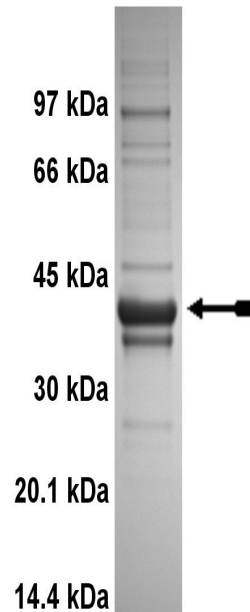
Quality Control Testing

Kinase Assay: 8.6–86.6ng 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 47% amino acid coverage of 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.



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 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l 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 8.6–86.6ng 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 5 μ l of 5 x reaction buffer to wells.
2. Add 2.5 μ l of **(KKKSPGEYVNIEFG)**.
3. Add **2.5 μ l (8.6–86.6ng) TRKA, active**.
4. Add 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 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.

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:

```
1  MHHHHHHEFN  KCGRRNKFGEI  NRPAVLAPED  GLAMSLHFMT  LGGSSLSPT  GKGSGLQGHI
61  IENPQYFSDA  CVHHIKRRDI  VLKWELEGGA  FGKVFLEACH  NLLPEQDKML  VAVKALKEAS
121  ESARQDFQRE  AELLTMLQHQ  HIVRFFGVCT  EGRPLLMVFE  YMRHGDLNRF  LRSHGPDACL
181  LAGGEDVAPG  PLGLGQLLAV  ASQVAAGMVY  LAGLHFVHRD  LATRNCLVGQ  GLVVKIGDFG
241  MSRDIYSTDY  YRVGGRTMLP  IRWMPPEPES  YRKFTTESDV  WSFGVVLWEI  FTYGKQPWYQ
301  LSNTAIDCI  TQGRELERPR  ACPPEVYAIM  RGCWQREPQQ  RHSIKDVHAR  LQALAQAPPV
361  YLDVLG
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Recombinant TRKA nucleotide sequence:

```
1  atgcatcattc  accatcacca  tgaattcaac  aaatgtggac  ggagaaaca  gtttgggatc
61  aaccgcccgg  ctgtgctggc  tccagaggat  gggctggcca  tgtccctgca  tttcatgaca
121  ttgggtggca  gctccctgtc  cccaccgag  ggcaaaggct  ctgggctcca  aggccacatc
181  atcgagaacc  cacaataactt  cagtgatgcc  tgtgttcacc  acatcaagcg  cggggacatc
241  gtgctcaagt  gggagctggg  ggagggcgcc  tttgggaagg  tcttccttgc  tgagtgccac
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421  cacatcgtgc  gcttcttcgg  cgtctgcacc  gagggccgcc  cctgtctcat  ggtctttgag
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