

## Product Information

**TRKA (440-end), active, GST-tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 cells**

Catalog Number **T4202**  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

Synonyms: NTRK1, MTC, TRK, pl 40-TrkA, DKFZp781114186

### Product Description

TRKA is a member of the trk proto-oncogene family and encodes a 140 kDa, membrane-spanning protein tyrosine kinase that is the functional receptor for nerve growth factor (NGF). NGF elicits the rapid phosphorylation of gp140trk on tyrosine residues leading to increased c-Fos expression, DNA synthesis, and morphologic transformation.<sup>1</sup> A decreased expression of TRKA on the striatal cholinergic neurons has been observed which may contribute, when it reaches a crucial threshold, to the death of cholinergic neurons observed in Alzheimer's disease.<sup>2</sup>

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 002529. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: 66 kDa

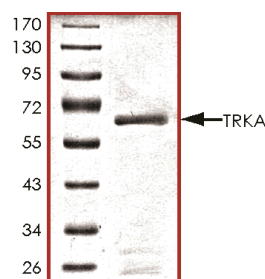
### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

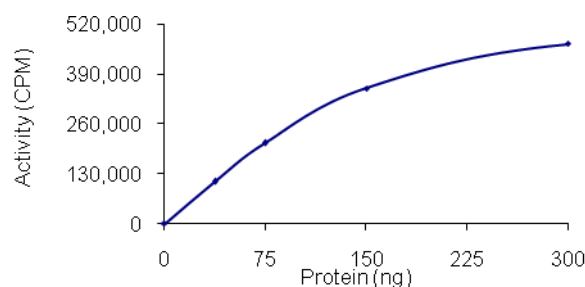
### Storage/Stability

The product ships on dry ice and storage at  $-70\text{ }^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of a Typical Lot  
 $\geq 70\%$  (SDS-PAGE, densitometry)



**Figure 2.**  
Specific Activity of a Typical Lot  
19–25 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 20 mM  $\text{MgCl}_2$ , 25 mM  $\text{MnCl}_2$ , 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/ $\mu\text{l}$  BSA solution.

Kinase Solution – Dilute the active TRKA (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. **Note:** The Specific Activity of a Typical Lot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active TRKA kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-<sup>32</sup>P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-<sup>32</sup>P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate Poly (Glu:Tyr, 4:1) in water at a final concentration of 2 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

#### Kinase Assay

This assay involves the use of the <sup>32</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active TRKA, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-<sup>32</sup>P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
  - 10 µl of Kinase Solution
  - 10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-<sup>32</sup>P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5 µl of the γ-<sup>32</sup>P-ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

#### Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 } \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)  
nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

Δcpm = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

#### References

1. Kaplan, D R. et al., The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science*, **252**, 554-558 (1991).
2. Boissiere, F. et al., Neurotrophin receptors and selective loss of cholinergic neurons in Alzheimer disease. *Mol. Chem. Neuropathol.*, **28**, 219-223 (1996).

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