

## TRKA (F589L), Active

Recombinant human protein expressed in Sf9 cells

**Catalog # N16-12EG**

Lot # D2494-9

### Product Description

Recombinant human TRKA (F589L) (440-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_002529](#).

### Gene Aliases

NTRK1; MTC; TRK; TRK1; p140-TrkA; DKFZp78114186

### Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

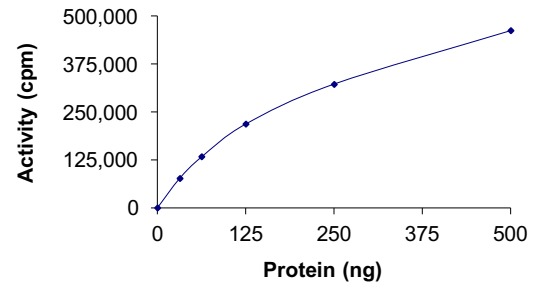
### Scientific Background

TRKA is a member of the trk proto-oncogene family and encodes a 140kDa, 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 (1). 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 disease (2).

### References

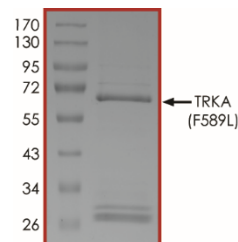
1. Kaplan, D R. et al: The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. Science. 1991 Apr 26;252(5005):554-8.
2. Boissiere, F. et al: Neurotrophin receptors and selective loss of cholinergic neurons in Alzheimer disease. Mol Chem Neuropathol. 1996 May-Aug;28(1-3):219-23.

### Specific Activity



The specific activity of TRKA (F589L) was determined to be **116 nmol /min/mg** as per activity assay protocol.

### Purity



The purity was determined to be **>85%** by densitometry. Approx. MW **66kDa**.

## TRKA (F589L), Active

Recombinant human protein expressed in Sf9 cells

|                    |   |
|--------------------|---|
| Catalog #          | N16-12EG  |
| Specific Activity  | 116 nmol/min/mg   |
| Lot #              | D2494-9   |
| Purity             | >85%  |
| Concentration      | 0.1µg/µl  |
| Stability          | 1yr at -70°C from date of shipment  |
| Storage & Shipping | Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice. |

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: N16-12EG)

Active TRKA (F589L) (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active TRKA (F589L) for optimal results).

### Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50 ng/µl BSA solution.

### Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl<sub>2</sub>, 25mM MnCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: P61-58)

Poly (Glu:Tyr, 4:1) synthetic peptide substrate diluted in Tris-HCl buffer (pH 7.5) to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active TRKA (F589L), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  - Component 1.** 10µl of diluted Active TRKA (F589L) (Catalog #N16-12EG)
  - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #P61-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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