TRKC (G623R), Active
Recombinant human protein expressed in Sf9 cells
Catalog # N18-12CH
Lot # H2775-9

Product Description
Recombinant human TRKC (G623R) (507-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_001012338.

Gene Aliases
NTRK3, gp145 (trkC)

Formulation
Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, and 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
TRKC is a member of the TRK family of tyrosine kinase receptors and is the high affinity catalytic receptor for the neurotrophin NT-3 (neurotrophin-3). TRKC mediates the multiple cellular effects of the NT-3 neurotrophic factor, which includes neuronal differentiation and survival (1). TRKC has been implicated in insulin signaling pathway through interactions with the MUSK protein receptor and the VEGF receptor. Mutations in the TRKC gene have been associated with medulloblastomas, secretory breast carcinomas and other cancers (2).

References

Specific Activity
The specific activity of TRKC (G623R) was determined to be 12 nmol/min/mg as per activity assay protocol.

Purity
The purity of TRKC was determined to be >70% by densitometry, approx. MW 39kDa.

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Activity Assay Protocol

**Reaction Components**

**Active Kinase** (Catalog #: N18-12CH)
Active TRKC (G623R) (0.05µg/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 TRKC 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 final 50ng/µl BSA solution.

**Kinase Assay Buffer II** (Catalog #: K02-09)
Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**[³³P]-ATP Assay Cocktail**
Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 (4:1 Glu, Tyr) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

**Assay Protocol**

1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
2. Thaw the Active TRKC (G623R), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
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 TRKC (G623R) (Catalog #N18-12CH)
   - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #P61-58)
   - Component 3. 5µl distilled H₂O (4°C)
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 H₂O.
5. Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
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 H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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 [P³³]-ATP Specific Activity (SA) (cpm/pmol)**

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

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

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

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