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**TFG-TRKA (TRK-T3), Active**
Recombinant human protein expressed in Sf9 cells

**Catalog # N16-19CG**
**Lot # K1779-1**

**Product Description**
Recombinant human fusion TFG (1-193)-TRKA (399-end) protein was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of KIAA1549 is NM_001195478 and BRAF is NM_002529.

**Gene Aliases**

TFG:  HMSNP; SPG57; TF6
TRKA:  NTRK1, MTC, TRK, p140-TrkA

**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**
TFG-TRKA (TRK-T3) is a chimeric cytoplasmic oncoprotein isolated from a papillary thyroid tumor. This oncoprotein is the consequence of a t(1, 3) translocation juxtaposing the tyrosine kinase domain of the NTRK1 receptor to the N-terminal portion of a protein encoded by TFG (TRKFused gene), a novel gene first discovered in the rearranged form. TRK-T3 fusion oncoprotein displays a constitutive tyrosine kinase activity resulting in its capability to transform mouse NIH3T3 cells. The TFG portion of TRK-T3 contains a coiled-coil domain most likely responsible for the constitutive, ligand-independent activation of the receptor tyrosine kinase activity through TRK-T3 oligomerization.

**References**

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**Specific Activity**

The specific activity of TFG-TRKA (TRK-T3) was determined to be **16 nmol/min/mg** in a coupled assay as per activity assay protocol.

**Purity**

The purity of TFG-TRKA (TRK-T3) protein was determined to be **>80%** by densitometry, approx. MW **100 kDa**.

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**TFG-TRKA (TRK-T3), Active**
Recombinant human protein expressed in Sf9 cells

**Catalog # N16-19CG**
**Specific Activity** 16 nmol/min/mg
**Lot #** K1779-1
**Purity** >80%
**Concentration** 0.1 µg/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.
Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: N16-19CG)
Active TFG-TRKA (TRK-T3) (0.1 µg/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 TFG-TRKA (TRK-T3) 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.

Substrate (Catalog #: P61-58)
Poly (Glu₄, Tyr₁) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

[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 I (Catalog #: K01-09). Store 200µl aliquots at −20°C.

Assay Protocol

Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active TFG-TRKA (TRK-T3), 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 TFG-TRKA (TRK-T3) (Catalog # N16-19CG)
   - Component 2. 5µl of 1 mg/ml stock solution of substrate (Catalog #P61-58)
   - Component 3. 5µl distilled H₂O (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 H₂O.
Step 5. Initiate the reaction by the addition of 5µl [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.
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 H₂O) 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 (cpm) 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 [³³P]-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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