

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

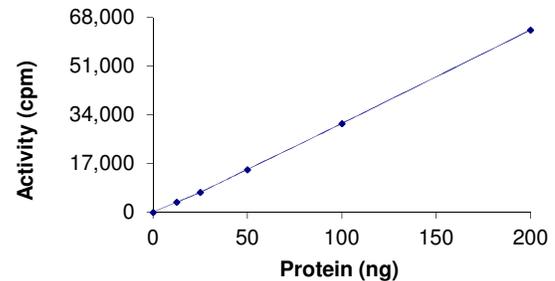
TFG-TRKA (TRK-T3) is a chimeric cytoplasmic oncoprotein isolated from a papillary thyroid tumor. This oncoprotein is the consequence of a t(1, 3) translocation juxtaposing the tyrosine kinase domain of the NTRK1 receptor to the N-terminal portion of a protein encoded by TFG (TRK-fused gene), a novel 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

1. Greco A. et al: The DNA rearrangement that generates the TRK-T3 oncogene involves a novel gene on chromosome 3 whose product has a potential coiled-coil domain. *Mol Cell Biol.* 15(11):6118-27, 1995.
2. Greco A, et al. Role of the TFG N-terminus and coiled-coil domain in the transforming activity of the thyroid TRK-T3 oncogene. *Oncogene.* 12;16(6):809-16, 1998.

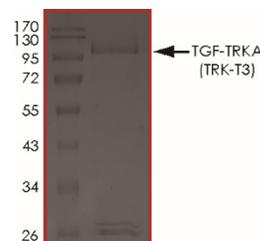
To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

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**.

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) (0.1µg/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 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.

[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.

Substrate (Catalog #: P61-58)

Poly (Glu₄Tyr₁) synthetic peptide substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

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)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.