Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ TRK-C

Neurotrophic tyrosine kinase, receptor, type 3

Recombinant Human Active Protein Kinase

HGNC Symbol: NTRK3

Synonyms: Gp145-TrkC

Product No.: 0775-0000-1

Lot: 005

Description: Human TRK-C, C-terminal fragment, amino acids V_{510}-G_{825} (as in NCBI/Protein entry NP_002521.2), activated, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: TRK-C Lot 005, was confirmed as TRK-C by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 64,870 Da

Expression: Baculovirus infected Sf9 cells

Purification: GST-Affinity Chromatography

Activation: in vitro autoactivation

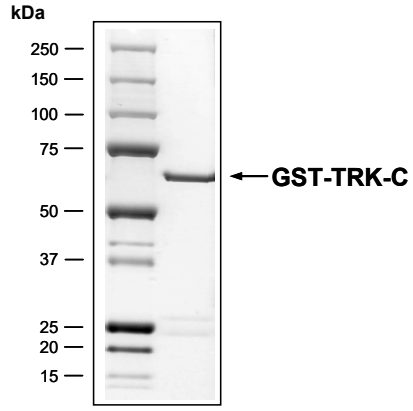
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.085 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

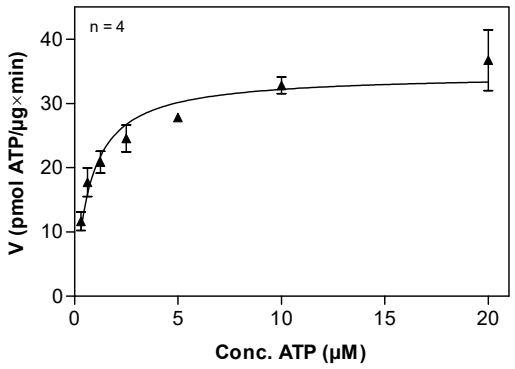
Biochemical Parameters:
Specific kinase activity (P_i transfer): 35 pmol/µg×min
ATP-K_M: 0.7 µM

TRK-C Lot 005:
Coomassie stain



2.0 µg GST-TRK-C

TRK-C Lot 005:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg / ml $PEG_{20.000}$
  ATP (variable)
  Substrate: $Poly(Glu:Tyr)_{4:1}$
  (Sigma P-0275), 40 µg / ml
  TRK-C: 0.4 µg / ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology: TRK-C Lot 005
was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ TRK-C

Product No.: 0775-0000-1

TRK-C Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
 241  PLAMFVIENP QYFRQGHNCH KPDTYVQHIK RRDIVLKREL GEGAFGKVFL AECYNLSPTK 300
 301  DKMLVAVKAL KDPTLAARKD FQREAELLTN LQHEHIVKFY GVCGDGDPLI MVFEYMKHGD 360
 361  LNKFLRAHGP DAMILVDGQP RQAKGELGLS QMLHIASQIA SGMVYLASQH FVHRDLATRN 420
 421  CLVGANLLVK IGDFGMSRDV YSTDYYRVGG HTMLPIRWMP PESIMYRKFT TESDVWSFGV 480
 481  ILWEIFTYGK QPWFQLSNTE VIECITQGRV LERPRVCPKE VYDVMLGCWQ REPQQRLNIK 540
 541  EIYKILHALG KATPIYLDIL G                                          600
```

1-218: GST **Red:** HIS6-tag **Green**: 3C cleavage site **blue**:TRK-C fragment

TRK-C wt[1] amino acid sequence

```
   1  MDVSLCPAKC SFWRIFLLGS VWLDYVGSVL ACPANCVCSK TEINCRRPDD GNLFPLLEGQ  60
  61  DSGNSNGNAS INITDISRNI TSIHIENWRS LHTLNAVDME LYTGLQKLTI KNSGLRSIQP 120
 121  RAFAKNPHLR YINLSSNRLT TLSWQLFQTL SLRELQLEQN FFNCSCDIRW MQLWQEQGEA 180
 181  KLNSQNLYCI NADGSQLPLF RMNISQCDLP EISVSHVNLT VREGDNAVIT CNGSGSPLPD 240
 241  VDWIVTGLQS INTHQTNLNW TNVHAINLTL VNVTSEDNGF TLTCIAENVV GMSNASVALT 300
 301  VYYPPRVVSL EEPELRLEHC IEFVVRGNPP PTLHWLHNGQ PLRESKIIHV EYYQEGEISE 360
 361  GCLLFNKPTH YNNGNYTLIA KNPLGTANQT INGHFLKEPF PESTDNFILF DEVSPTPPIT 420
 421  VTHKPEEDTF GVSIAVGLAA FACVLLVVLF VMINKYGRRS KFGMKGPVAV ISGEEDSASP 480
 481  LHHINHGITT PSSLDAGPDT VVIGMTRIPV IENPQYFRQG HNCHKPDTYV QHIKRRDIVL 540
 541  KRELGEGAFG KVFLAECYNL SPTKDKMLVA VKALKDPTLA ARKDFQREAE LLTNLQHEHI 600
 600  VKFYGVCGDG DPLIMVFEYM KHGDLNKFLR AHGPNAMILV DGQPRQAKGE LGLSQMLHIA 660
 661  SQIASGMVYL ASQHFVHRDL ATRNCLVGAN LLVKIGDFGM SRDVYSTDYY RVGGHTMLPI 720
 721  RWMPPESIMY RKFTTESDVW SFGVILWEIF TYGKQPWFQL SNTEVIECIT QGRVLERPRV 780
 781  CPKEVYDVML GCWQREPQQR LNIKEIYKIL HALGKATPIY LDILG                 840
```

blue: TRK-C sequence expressed in fusionprotein

[1]NCBI/Protein accession number NP_002521.2

Recombinant Proteins