Certificate of Analysis

REACTION BIOLOGY

ProQinase™ RBER-NTRK3tide

Recombinant Protein Kinase Substrate

Product No.: 1132-0000-1

Lot: 011

Description: Artificial fusion protein consisting of an N-terminal GST-tag separated by a Thrombin cleavage site from a fragment of the human retinoblastoma protein RB1, amino acids S_{773}-K_{928} (as in NCBI/Protein entry NP_000312.2) followed by 11 Arg residues (ER) and a peptide sequence (VYSTDYYRLFNPS), derived from the human NTRK3 protein (NCBI/Protein entry NP_001012338.1, aa V_{704}-S_{716}).

Theoretical $MW_{Fusion\ Protein}$: 47,450 Da

Expression host: E.coli

Purification: GST-Affinity and ion exchange chromatography

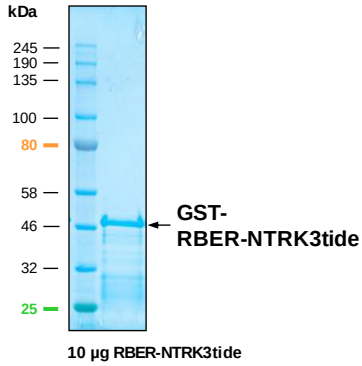
ATPase activity: In an ADP-Glo™ assay (Promega) with 10 µM ATP or 30 µM ATP, the ATP → ADP conversion within 30 min is < 1% at a concentration of 100 µg/ml substrate.
Detailed ATPase assay conditions on request

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 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: 2.325 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

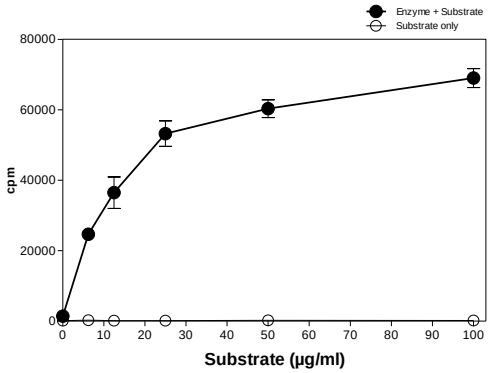
RBER-NTRK3tide Lot 011:
Coomassie stain



10 µg RBER-NTRK3tide

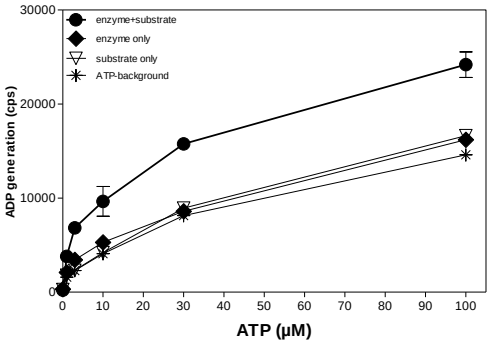
Phosphorylation of RBER-NTRK3tide by TRK-C

Radiometric filter binding assay



Assay conditions:
70 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: 1 µM
Substrate: variable concentration
Kinase: 1 µg/ml
MSFC membrane (Millipore)

ADP-Glo™ assay (Promega)



Assay conditions:
70 mM HEPES-NaOH, pH 7.5
3 mM MgCl2
3 mM MnCl2
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml PEG20.000
ATP: variable concentration
1 % (v/v) DMSO
Substrate: 100 µg/ml
Kinase: 1 µg/ml

Recombinant Proteins

ProQinase™ RBER-NTRK3tide

Product No.: 1132-0000-1

RBER-NTRK3tide Recombinant Fusion Protein Amino Acid Sequence

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  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 LVPRGSPEFS TRPPTLSPIP 240
241  HIPRSPYKFP SSPLRIPGGN IYISPLKSPY KISEGLPTPT KMTPRSRILV SIGESFGTSE 300
301  KFQKINQMVC NSDRVLKRSA EGSNPPKPLK KLRFDIEGSD EADGSKHLPG ESKFQQKLAE 360
361  MTSTRTRMQK QKMNDSMDTS NKEEKRRRRR RRRRRRVYST DYYRLFNPS            420
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1-218: GST **Pink**: Thrombin cleavage site **Green**: R_{11}-sequence **blue**: RB1 fragment **boxed**: NTRK3tide sequence

Recombinant Proteins