

ProQinase™ RBER-NTRK3tide

generic protein kinase substrate

Recombinant Protein Kinase Substrate

Product No.: 1132-0000-1

Lot: 019

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 S₇₇₃-K₉₂₈ (as in [NCBI/Protein](#) entry NP_000312.2) followed by 11 Arg residues (ER) and a peptide sequence (VYSTDYRLFNPS), derived from the human NTRK3 protein ([NCBI/Protein](#) entry NP_001012338.1, aa V₇₀₄-S₇₁₆).

Theoretical MW_{Fusion Protein}: 47450 Da

Expression host: E.coli

Purification: GST-Affinity 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

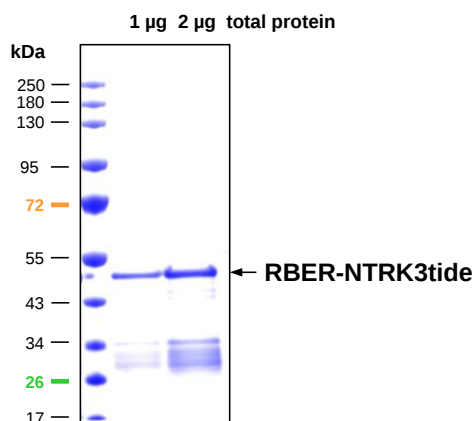
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 10 % glycerol

Storage temperature: -80°C

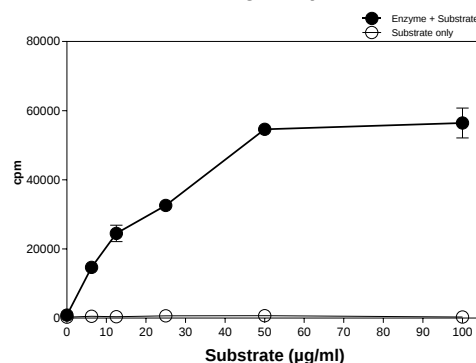
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.528 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Substrate LOT019:
Coomassie stain



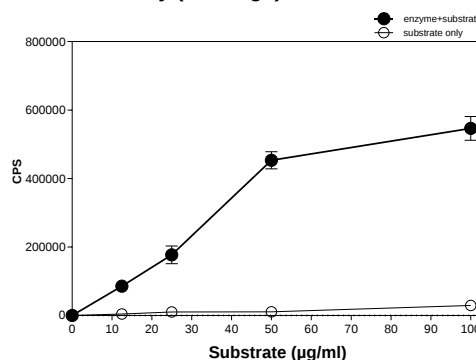
Phosphorylation of RBER-NTRK3tide by TRKC Radiometric filter binding assay



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 PEG_{20,000}
ATP: 1 µM
Substrate: variable concentration
Kinase: 0.5 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 PEG_{20,000}
ATP: variable concentration
1 % (v/v) DMSO
Substrate: 100 µg/ml
Kinase: 0.5 µg/ml

Sequence information

RBER-NTRK3tide Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSP	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQQLAE	360
361	MTSTRTRMQ	QKMNDSDMTS	NKEEKRRRR	RRRRRVYST	DYYRLFNPS		420

1-218: GST Pink: Thrombin cleavage site Green: R11-sequence blue: RB1 fragment boxed: NTRK3tide sequence

Recombinant Proteins