

# RBER-NTRK3tide

Recombinant Protein Kinase Substrate

Product No.: 1132-0000-1

Lot: 003

**Description:** Artificial fusion protein consisting of a N-terminal GST-tag separated by a Thrombin cleavage site from a fragment of the human retinoblastoma protein RB1, amino acids S<sub>773</sub>-K<sub>928</sub> (as in NCBI/Protein entry NP\_000312.2) followed by 11 Arg residues (ER) and a peptide sequence (VYSTDYRFLNPS), derived from the human NTRK3 protein (GenBank entry Q16288, aa V<sub>704</sub>-S<sub>716</sub>).  
Expressed in E.coli.

**Theoretical MW:** 47,450 Da

**Expression:** E. coli

**Purification:** Affinity chromatography using GSH-agarose, followed by ion exchange chromatography

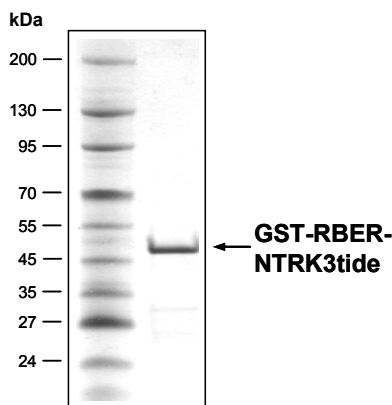
**ATPase activity:** In an ADP-Glo™ assay (Promega) with 10 μM ATP or 30 μM ATP, the ATP → ADP conversion within 30 min is below 1% at a concentration of 100 μg/ml RBER-NTRK3tide\*  
\*detailed ATPase assay conditions on request

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 15 mM reduced glutathione, 10% glycerol

**Storage temperature:** -80°C  
Avoid repeated freeze-thaw cycles!

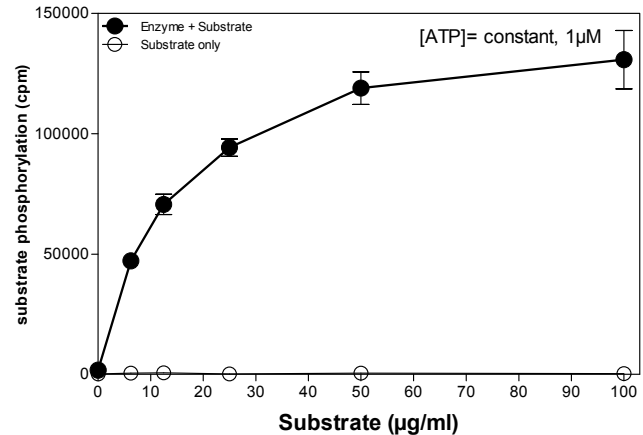
**Protein concentration:** 0.954 μg/μl (Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**Coomassie stain:**



2.0 μg GST-RBER-NTRK3tide

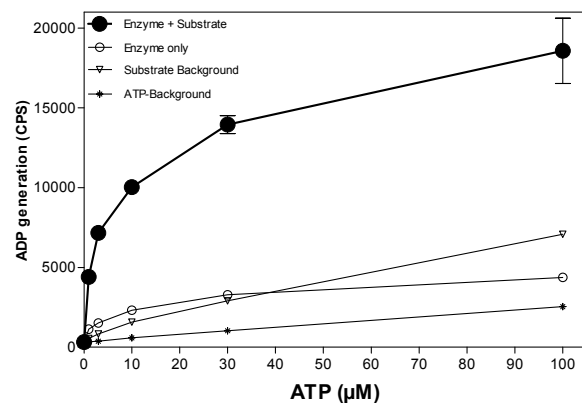
## Phosphorylation of RBER-NTRK3tide by the kinase TRK-C (Radiometric filter binding assay):



Assay mixture:

70 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20,000</sub>  
ATP: 1 μM  
Substrate RBER-NTRK3tide: variable concentration  
TRK-C: 800 ng/ml  
MSFC membrane (Millipore)

## Phosphorylation of RBER-NTRK3tide by the kinase TRK-C (ADP-Glo™ assay / Promega):



Assay mixture:

70 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20,000</sub>  
ATP: variable concentration  
1% (v/v) DMSO  
Substrate RBER-NTRK3tide: 100 μg/ml  
TRK-C: 1.0 μg/ml

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