Certificate of Analysis

ProQinase
TARGETING CANCER

RBER-NTRK3tide

Recombinant Protein Kinase Substrate

Product No.: 1132-0000-1

Lot: 002

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_{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 (GenBank entry Q 16288, aa V_{704}-S_{716}). Expressed in E.coli.

Theoretical MW: 47,450 Da

Expression: E. coli

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

ATPase acticity: In an ADP-Glo^{TM} 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

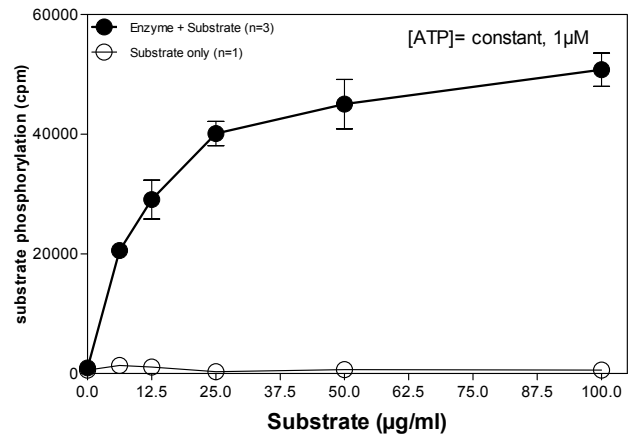**Storage temperature:** -80°C
Avoid repeated freeze-thaw cycles!

Protein concentration: 1.010 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:
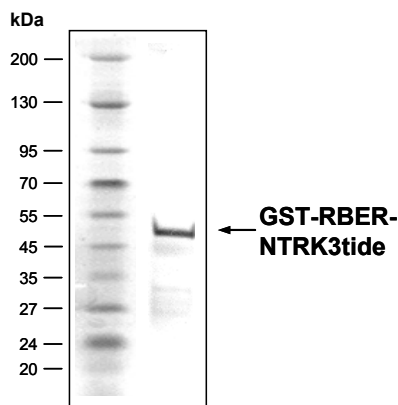

3.0 µg GST-RBER-NTRK3tide

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$_2$
3 mM MnCl$_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml PEG$_{20.000}$
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^{TM} assay / Promega):
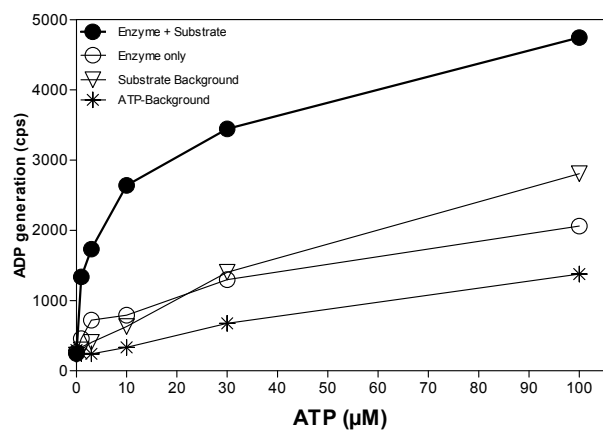
Assay mixture:

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 RBER-NTRK3tide: 100 µg/ml
TRK-C: 800 ng/ml