

RBER-IRStide

Recombinant Protein Kinase Substrate

Product No.: 0863-0000-1

Lot: 033

Description: Artificial fusion protein consisting of an N-terminal GST-tag separated by a Thrombin cleavage site from a fragment of the human RB1 protein, amino acids S₇₇₃-K₉₂₈ (as in [NCBI/Protein](#) entry NP_000312.2) followed by 11 Arg residues (ER) and a peptide sequence (HTDDGYMPMSPGVA, IRStide). Expressed in E.coli.

Theoretical MW_{Fusion Protein}: 48,559 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 approx. 1% at a concentration of 100 μg/ml substrate.
Detailed ATPase assay conditions on request

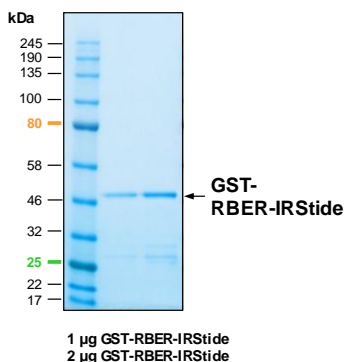
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C

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

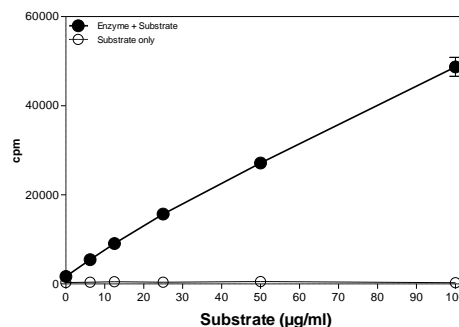
Protein concentration: 0.465 μg/μl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

RBER-IRStide Lot 033: Coomassie stain



Phosphorylation of RBER-IRStide by p38-gamma

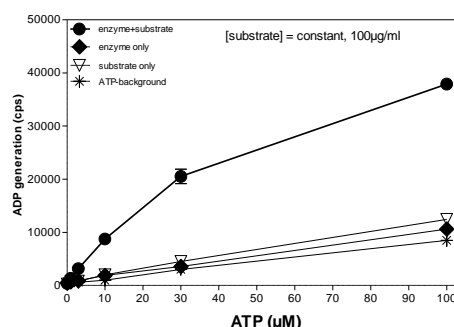
Radiometric filter binding assay



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: 2 μg/ml
MSFC membrane (Millipore)

ADP-Glo™ assay (Promega)



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 (RBER-IRStide): 100 μg/ml
Kinase: 2 μg/ml

This product was manufactured at ProQinase in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals. ProQinase disclaims any warranty explicitly or implied that the use of the product or parts of the product is free from third party intellectual property claims unless this is explicitly stated.

RBER-IRStide

Product No.: 0863-0000-1

RBER-IRStide Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQG WQATF	GGGDHPPKSD	LVPRGSP EFS	TRPPTLSPI P	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQ MVC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP G	ESKFQQLAE	360
361	MTSTRTRMQ K	QKMND SMDTS	NKEEKRRRRR	RRRRR R	KKHT	DDGYMPMSPG	420

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

This product was manufactured at ProQinase in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals. ProQinase disclaims any warranty explicitly or implied that the use of the product or parts of the product is free from third party intellectual property claims unless this is explicitly stated.