

RBER-IRStide

Recombinant Protein Kinase Substrate

Product No.: 0863-0000-1

Lot: 030

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}: 47,559 Da

Expression host: E.coli

Purification: GST-affinity chromatography, 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 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

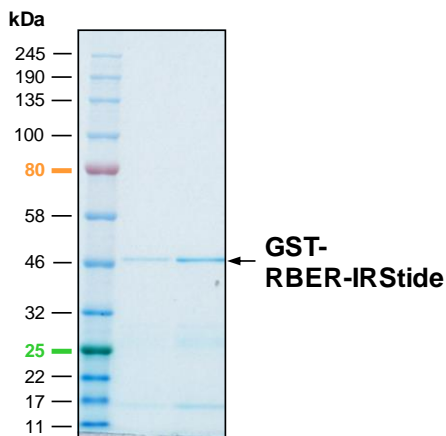
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10μl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.727 μg/μl

(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

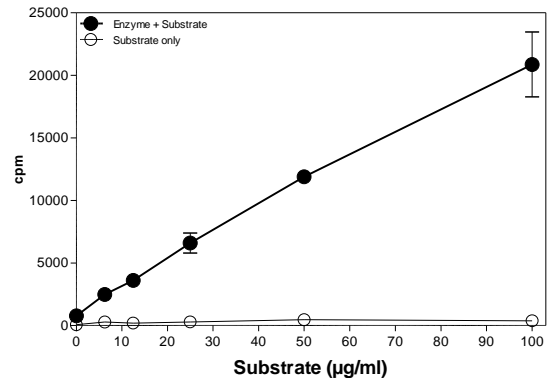
RBER-IRStide Lot030:

Coomassie stain



1 μg GST-RBER-IRStide
2 μg GST-RBER-IRStide

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}

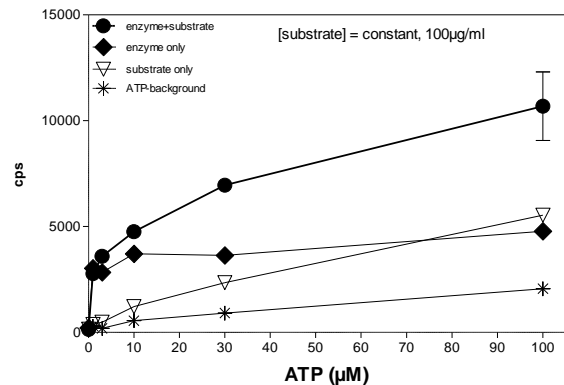
ATP: 1 μM

Substrate: variable concentration

Kinase: 2.0 μg/ml

MSFC membrane (Millipore)

Phosphorylation of RBER-IRStide by p38-gamma (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: 2.0 μg/ml

RB1-IRStide

Product No.: 0863-0000-1

RB1-IRStide Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSP	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRLIV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQQLAE	360
361	MTSTRTRMQK	QKMNSMDTS	NKEEKRRRRR	RRRRRKKHT	DDGYMPSPG	VA	420

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