

RBER-IRStide

Recombinant Protein Kinase Substrate

Product No.: 0863-0000-1

Lot: 040

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 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

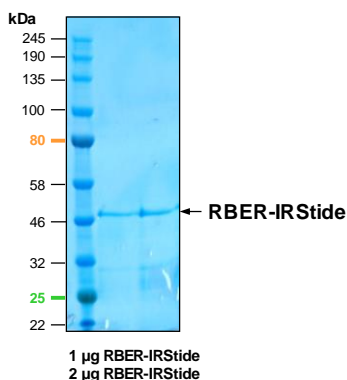
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

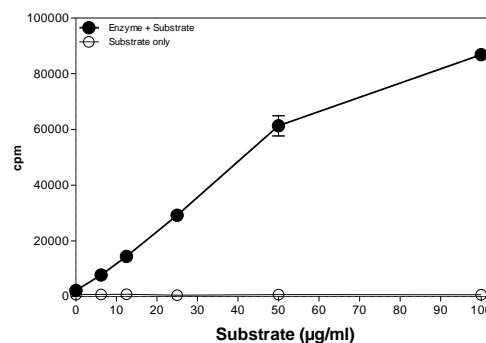
Protein concentration: 0.295 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

RBER-IRStide Lot 040: Coomassie stain



Phosphorylation of RBER-IRStide

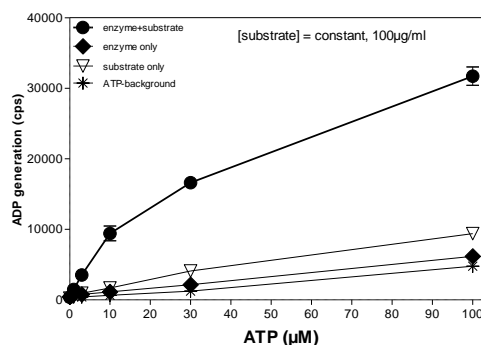
Radiometric filter binding assay



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 (p38-gamma): 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 (CDK6-CycD3): 2 µg/ml

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RB ER-IRStide Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	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	KMTPRSRLV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRS	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQQLAE	360
361	MTSTRTRMQK	QKMNDSDMTS	NKEEKRRRR	RRRRRK	KKHT	DDGYMPMSPG	420

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

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