

ProQinase™ RBER-IRStide

Recombinant Protein Kinase Substrate

HGNC Symbol: n/a

Synonyms: n/a

Product No.: 0863-0000-1

Lot: 052

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}: 47559 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

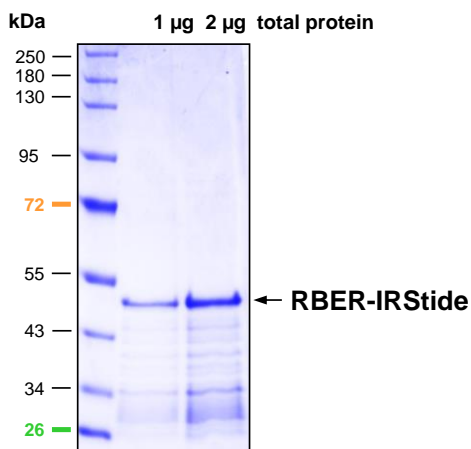
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 10 % glycerol

Storage temperature: -80°C

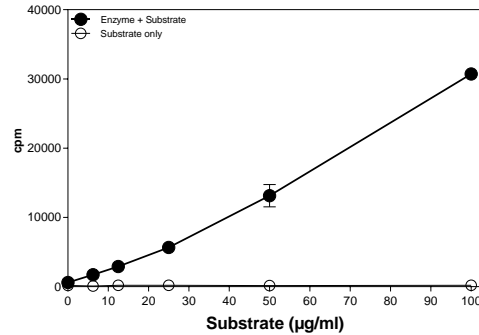
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10 μl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.313 μg/μl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

RBER-IRStide LOT052:
Coomassie stain



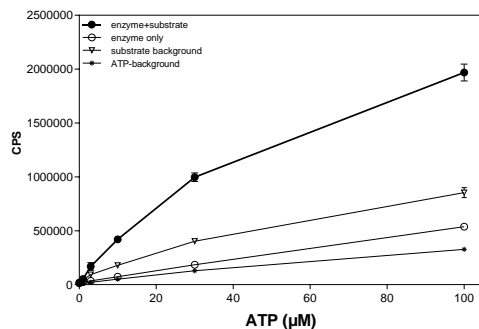
Phosphorylation of RBER-IRStide Radiometric filter binding assay



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)



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: variable concentration
1 % (v/v) DMSO
Substrate (RBER-IRStide): 100 μg/ml
Kinase (CDK6/CycD3): 2 μg/ml

Recombinant Proteins

Sequence information

Substrate Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDKVLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGDHPPKSD	LVPRGSP	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP	ESKFQOKLAE	360
361	MTSTRTRMQK	QKMNDSMDTS	NKEEKRRRRR	RRRRRKKHT	DDGYMPMSPG	VA	420

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

Recombinant Proteins

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