

## ProQinase™ 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 S<sub>773</sub>-K<sub>928</sub> (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<sub>Fusion Protein</sub>:** 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

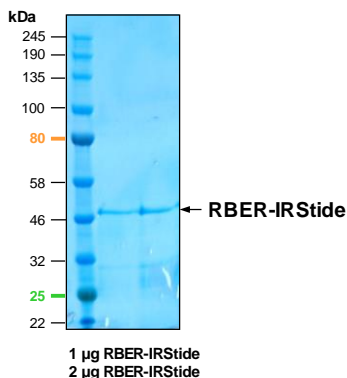
**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

**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!

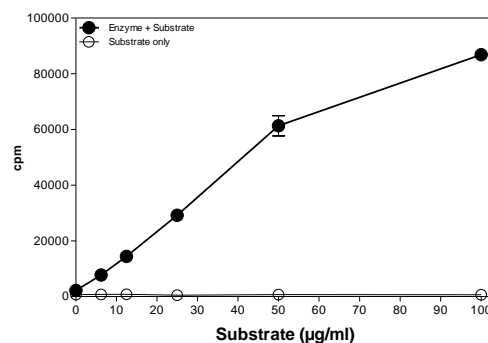
**Protein concentration:** 0.295 μg/μl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

**RBER-IRStide Lot 040:**  
**Coomassie stain**



### Phosphorylation of RBER-IRStide

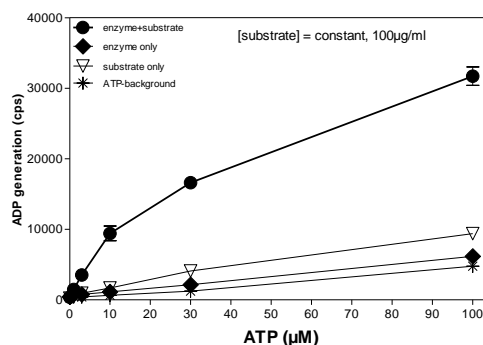
#### Radiometric filter binding assay



#### Assay conditions:

70 mM HEPES-NaOH, pH 7.5  
3 mM MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20,000</sub>  
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 MgCl<sub>2</sub>  
3 mM MnCl<sub>2</sub>  
3 μM Na-orthovanadate  
1.2 mM DTT  
50 μg/ml PEG<sub>20,000</sub>  
ATP: variable concentration  
1 % (v/v) DMSO  
Substrate (RBER-IRStide): 100 μg/ml  
Kinase (CDK6-CycD3): 2 μg/ml

This product was manufactured at Reaction Biology in Freiburg, Germany, and is for in vitro research use only, not for use in humans or animals.  
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## ProQinase™ RBER-IRStide

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RBER-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	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSP EFS	TRPPTLSPI P	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQ MVC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLP G	ESKFQQKLAE	360
361	MTSTRTRMQK	QKMNDSMDTS	NKEEKRRRRR	RRRRR RKKHT	DDGYMPMSPG	VA	420

1-218: GST **Pink**: Thrombin cleavage site **Green**: R<sub>11</sub>-sequence **blue**: RB1 fragment **boxed**: IRStide sequence