

ProQinase™ RBER-GSK3(14-27)

Recombinant Protein Kinase Substrate

Product No.: 0349-0000-5

Lot: 017

Description: Artificial fusion protein RBER-GSK3(14-27) consisting of a C-terminal fragment of the human retinoblastoma protein RB1 (GenBank entry NM_000321.1, aa S773-K928), fused to a R₁₁ spacer (ER) and a peptide sequence (SGRARTSSFAEPGG), derived from the human GSK3A protein (GenBank entry NM_019884.2, aa S14-G27). N-terminal GST fusion protein with a Thrombin cleavage site. Expressed in E.coli

Theoretical MW_{Fusion Protein}: 47,204 Da

Expression: E.coli

Purification: GSH-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 below 1% at a concentration of 100 μg/ml RBER-GSK3*.

*detailed ATPase assay conditions on request

Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 15 mM reduced glutathione, 10% glycerol

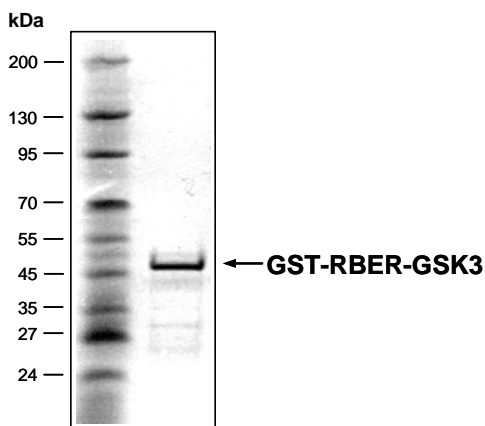
Storage temperature: -80°C

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: 0.754 μg/μl

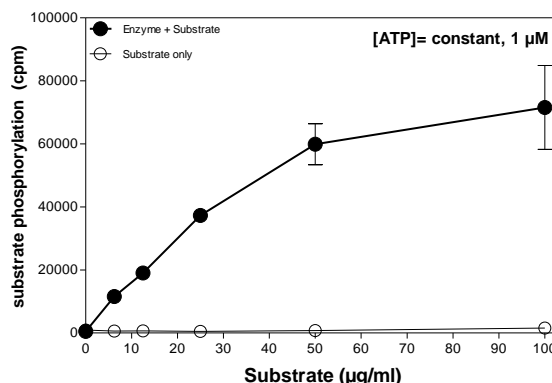
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



2.0 μg GST-RBER-GSK3

Phosphorylation of RBER-GSK3(14-27) by the kinase PIM1 (Radiometric filter binding assay):



Assay mixture:

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}

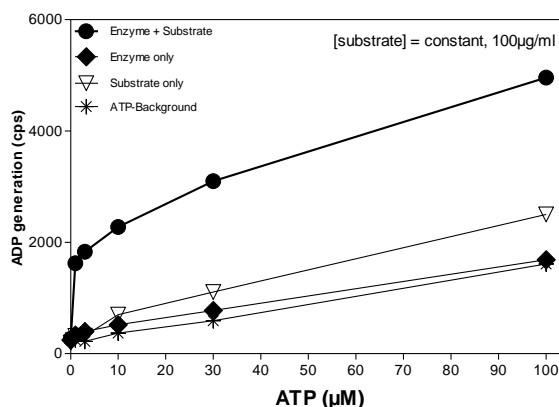
ATP: 1 μM

Substrate: RBER-GSK3(14-27) variable concentration

Enzyme: PIM1 400 ng/ml

MSFC membrane (Millipore)

Phosphorylation of RBER-GSK3(14-27) by the kinase PIM1 (ADP-Glo™ assay / Promega):



Assay mixture:

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-GSK3(14-27) 100 μg/ml

Enzyme: PIM1 2.0 μg/ml

Recombinant Proteins

ProQinase™ RBER-GSK3(14-27)

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RBER-GSK3(14-27) 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	VVLYMDEMCL	DAFPKLVCFK	180
181	KRIEAIPQID	KYLKSSKYIA	WPLQGQATF	GGGDHPPKSD	LVPRGSP EFES	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRIIV	SIGESFGTSE	300
301	KFQKINQVC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLPG	ESKFQQLAE	360
361	MTSTRTRMQK	QKMNSMDTS	NKEEKRRRRR	RRRRR SGRA	RTSSF AEPPG		420

1-218: GST **Pink:** Thrombin **blue:**RB1 fragment **Green:** R11-tag **shaded blue:** GSK3 peptide

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