

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

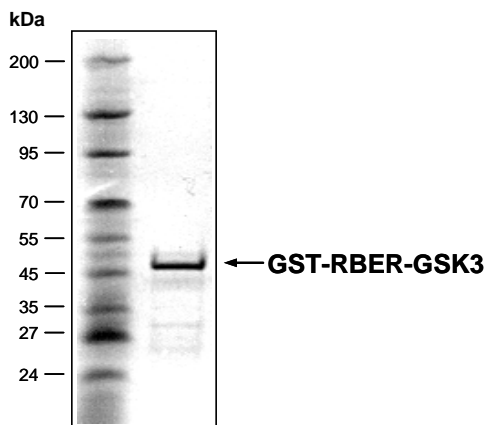
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 15 mM reduced glutathione, 10% glycerol

Storage temperature: -80°C

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

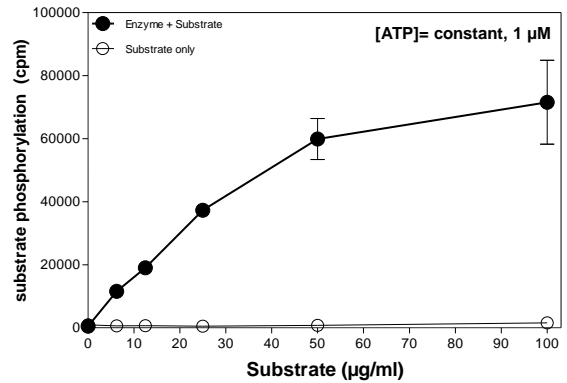
Protein concentration: 0.754 μg/μl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



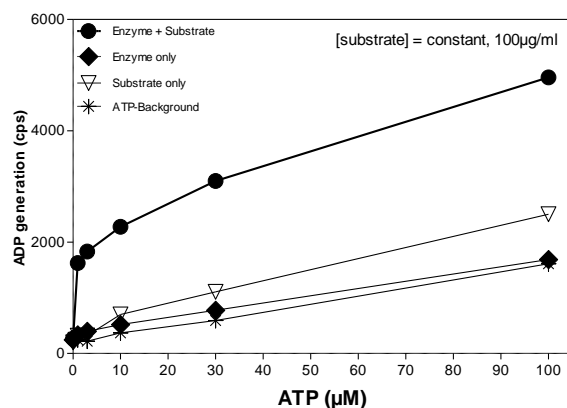
2.0 μg GST-RBER-GSK3

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

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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	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGQATF	GGGDHPPKSD	LVPRGS PEFS	TRPPTLSPIP	240
241	HIPRSPYKFP	SSPLRIPGGN	IYISPLKSPY	KISEGLPTPT	KMTPRSRILV	SIGESFGTSE	300
301	KFQKINQMC	NSDRVLKRSA	EGSNPPKPLK	KLRFDIEGSD	EADGSKHLPG	ESKFQOKLAE	360
361	MTSTRTRMOK	QKMND SMDTS	NKEEKRRRRR	RRRRRRSGRA	RTSSFAEPGG		420

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