

RBER-GSK3(14-27)

Recombinant Protein Kinase Substrate

Product No.: 0349-0000-5

Lot: 013

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: One-step affinity purification using GSH-agarose

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 RBER-GSK3*.

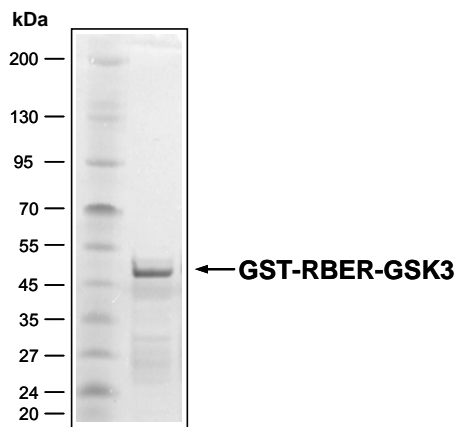
*detailed ATPase assay conditions on request

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

Storage temperature: -80°C
Avoid repeated freeze-thaw cycles!

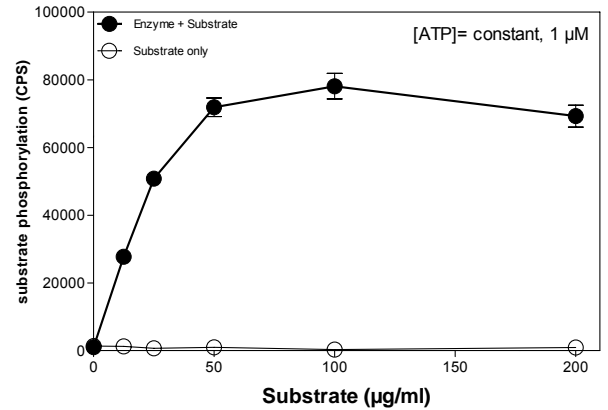
Protein concentration: 0.896 μg/μl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Coomassie stain:



2 μg GST-RBER-GSK3

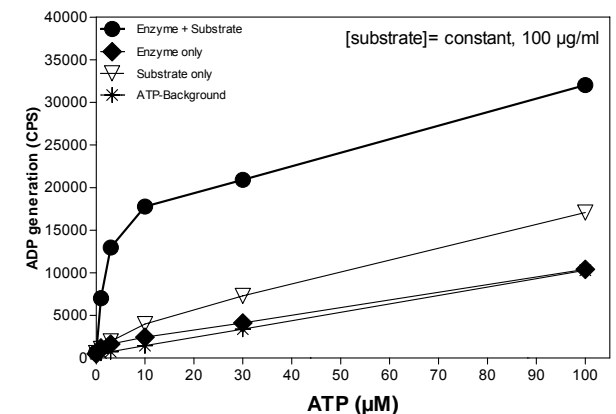
Phosphorylation of RBER-GSK3(14-27) by the kinase PIM1 (Radiometric filter binding assay):



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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