

Anti-phospho-Bim EL (Ser65)

(rabbit polyclonal IgG)

Catalog # 36-004

Lot # 24252

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 63-71 (C-PLAPPA[pS]PG) of rat phospho-Bim EL (Ser65). The immunizing sequence is identical in human and mouse.

Specificity: Recognizes phospho-Bim EL (Ser65), Mr ~48kDa. A non-specific protein was also detected, Mr ~66kDa, as well as a degradation product, Mr ~38kDa.

Species Cross-reactivity: Rat, mouse. Predicted to cross-react with human based on sequence homology.

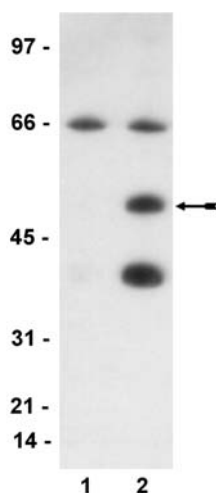
Formulation: 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 1.0µg/ml of this lot detected phospho-Bim EL (Ser65), using Bim-GST (Catalog # 12-483) that has been phosphorylated by JNK1α1/SAPK1c, active (Catalog # 14-327) in a cold kinase assay.



Immunoblot Analysis

Bim-GST (lane 1), and Bim-GST phosphorylated by JNK1α1/SAPK1c, active (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Bim EL (Ser65) (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-Bim EL (Ser65) (~48kDa).

Additional Research Applications

Immunoprecipitation-Immunoblotting: This antibody has been reported by an independent laboratory to detect phospho-Bim EL (Ser65) from mouse neuronal cell lysates.²

General Reference:

1. Putcha, G.V., *et al.*, Neuron **29**: 615-628, 2001.

Application Reference:

2. Putcha, G.V., *et al.*, Neuron **38**: 899-914, 2003.

Kinase Assay Protocol

Stock Solutions:

1. **Assay Dilution Buffer I** (ADBI, Catalog # 20-108): 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2. **Magnesium/ATP Cocktail** (Catalog 20-113): 500 μ M ATP and 75mM magnesium chloride in ADBI.
3. **Bim-GST** (Catalog # 12-483): Dilute with ADBI to 100ng/ μ l. Use 10 μ l per assay point.
4. **JNK1 α 1/SAPK1c, active** (Catalog # 14-327): Dilute with ADBI to 25ng/ μ l immediately prior to the assay. Use 10 μ l per assay point.

Assay Protocol:

1. Add 20 μ l of ADBI to a microcentrifuge tube.
2. Add 10 μ l (250ng) of **JNK1 α 1/SAPK1c**.
3. Add 10 μ l (1 μ g) of **Bim-GST**.
4. Add 10 μ l of the ATP solution.
5. Incubate for 30 minutes at 30°C.
6. Stop the reaction by adding 50 μ l of 2x SDS loading buffer.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 10 μ l of the sample per lane, and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
1. Block the blotted nitrocellulose in freshly prepared 2% BSA in TBS with 0.1% Tween 20 (TBST-BSA) for 60 minutes at room temperature with constant agitation.
2. Incubate the nitrocellulose with **1.0 μ g/ml of anti-phospho-Bim EL (Ser65)**, diluted in freshly prepared TBST-BSA overnight with agitation at 4°C.
3. Wash the nitrocellulose twice x 10 min. with 0.5% BSA in TBS with 0.1% Tween 20.
4. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-BSA for 1.5 hours at room temperature with agitation.
5. Wash the nitrocellulose twice x 10 min. with 0.5% BSA in TBS with 0.1% Tween 20.
6. Rinse the nitrocellulose in 4-5 changes of TBS.
7. Use detection method of choice (enhanced chemiluminescence was used).