

Certificate of Analysis

Anti-phospho-Bim EL (Ser65)

(rabbit polyclonal IgG)

Catalog # 36-004

Lot # JBC1370005

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.

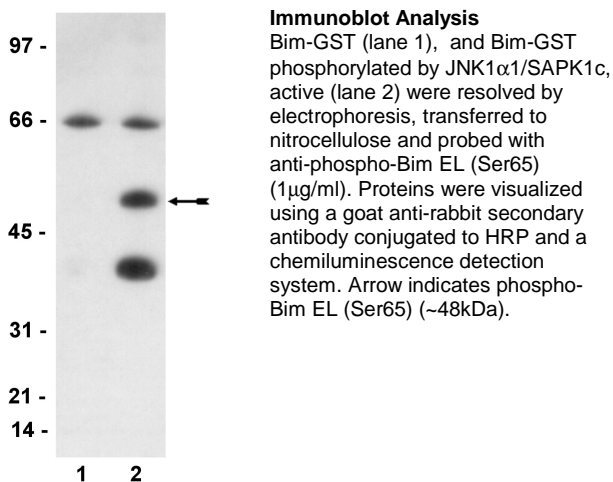
Formulation: 200µg of protein A purified rabbit IgG in 200µl of 0.014M phosphate buffer, pH 7.6, 0.175M NaCl, 0.07% sodium azide, and 30% glycerol. Liquid at -20°C.

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.



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 $^{\circ}$ 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 $^{\circ}$ 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).