



cell signaling solutions

Certificate of Analysis

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Anti-phospho-Bim EL (Ser55)

(rabbit polyclonal IgG)

Catalog # 36-005

Lot # 24253

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 55-63 (CPHG[pS]PQGP) of human phospho-Bim EL (Ser55). The immunizing sequence is identical in human, rat, and mouse.

Specificity: Recognizes phospho-Bim EL (Ser55), Mr ~48kDa. A degradation product, Mr ~38kDa was also detected.

Species Cross-reactivity: Human. Predicted to cross-react with rat and mouse based on sequence homology.

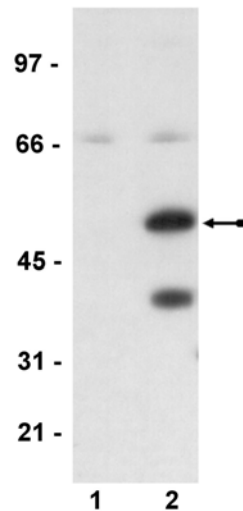
Formulation: 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

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 phosphorylated Bim EL (Ser55) using Bim-GST (Catalog # 12-483) previously 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 (Ser55) (1µg/ml). Proteins were visualized using a goat anti-rabbit antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-Bim (Ser55) (48kDa).

General References:

1. Putcha, G.V., *et al.*, *Neuron* **29**: 615-628, 2001.
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.
2. 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.
3. Incubate the nitrocellulose with **1.0 μ g/ml of anti-phospho-Bim EL (Ser55)**, diluted in freshly prepared TBST-BSA overnight with agitation at 4°C.
4. Wash the nitrocellulose twice for 10 minutes each with 0.5% BSA in TBS with 0.1% Tween 20.
5. 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.
6. Wash the nitrocellulose twice for 10 minutes each with 0.5% BSA in TBS with 0.1% Tween 20.
7. Rinse the nitrocellulose in 4-5 changes of TBS.
8. Use detection method of choice (enhanced chemiluminescence was used).