

## Certificate of Analysis

### Anti-phospho-Bad (Ser155), clone JBW101

(mouse monoclonal IgG<sub>2bλ</sub>)

Catalog # 05-628

Lot # 22024

**Immunogen:** KLH-conjugated, synthetic peptide (C-YGRELRRM[pS]DEFEG) corresponding to amino acids 147-160 of mouse Bad containing a N-terminal cysteine. The immunizing sequence is identical in rat and shares 12/14 identical amino acids in human. Ser155 in mouse corresponds to Ser156 in rat and Ser118 in human.

**Specificity:** Recognizes phosphorylated Bad, Mr 30kDa.

**Species Cross-reactivity:** Based on sequence homology, mouse, rat and may also cross-react with human.

**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.

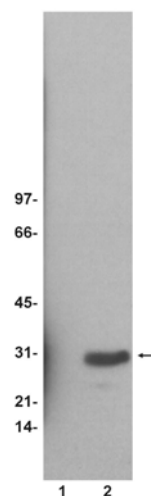
**Formulation:** 100μl of protein G purified mouse IgG<sub>2bλ</sub> in 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide, 5mg/ml BSA before the addition of glycerol to 30%. Liquid at -20°C.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoblot Analysis:** 1:500-1:5000 dilutions of this lot detected recombinant soluble murine Bad (Catalog # 14-357) phosphorylated at Ser155 by PKA (Catalog # 14-111).

**Kinase Assay:** PKA (Catalog #14-111) phosphorylated 3μg of soluble Bad (Catalog # 14-357). Subsequent immunoblot analysis detected the phosphorylated Bad using a 1:500-1:2000 dilution of this lot.



#### Immunoblot Analysis

200ng of recombinant Bad (Catalog #14-357) phosphorylated with PKA (Catalog #14-111), was resolved by electrophoresis, transferred to nitrocellulose and probed with a 1:5000 dilution of anti-phospho-Bad (Ser155). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Bad. Lane 1: recombinant soluble Bad untreated; Lane 2: recombinant soluble Bad, treated with PKA.

### Non-radioactive Kinase Assay Protocol

#### Stock Solutions:

1. **Assay Dilution Buffer I (ADBI)** (Catalog # 20-108): 20mM MOPS, pH 7.2, 25mM  $\beta$ -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2. **PKA, catalytic subunit** (Catalog # 14-114): Dilute with ADBI to 10ng/ $\mu$ l. Use 100ng per assay.
3. **Magnesium/ATP Cocktail:** 500 $\mu$ M cold ATP and 75mM magnesium chloride in ADBI.
4. **Bad, soluble:** Dilute with ADBI to 300ng/ $\mu$ l. Use 3 $\mu$ g per assay.

#### Assay Procedure:

1. Add 10 $\mu$ l (100ng) of PKA solution to an eppendorf tube
2. Add 10 $\mu$ l (3 $\mu$ g) of soluble Bad solution.
3. Add 50 $\mu$ l of Magnesium/ATP Cocktail.
4. Shake the reaction mixture for 30 minutes at 30°C.
5. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a sample of the supernatant and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
6. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200), and 0.2% Tween-20 (TBST-MLK) for 1 hour at 20-25°C with constant agitation.
7. Incubate the nitrocellulose with a **1:500 to 1:5000 dilution of anti-phospho-Bad (Ser155)**, in freshly prepared TBST-MLK for 3 hours at room temperature with agitation.
8. Wash the nitrocellulose twice with water.
9. Incubate the nitrocellulose in the secondary reagent of choice (goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in TBST-MLK for 1 hour at room temperature with agitation.
10. Wash the nitrocellulose with water twice.
11. Wash the nitrocellulose in TBS-0.1% Tween 20 for 5 minutes.
12. Rinse the nitrocellulose in 4-5 changes of water, 10 minutes each.
13. Use detection method of choice (enhanced chemiluminescence was used).