



cell signaling solutions

## Certificate of Analysis

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### Anti-Bcl2, clone 100

(mouse monoclonal IgG<sub>1</sub>)

Catalog # 05-729

Lot # 26504

**Immunogen:** Synthetic peptide corresponding to amino acids 41-54 of human Bcl2 protein.

**Specificity:** Bcl2, Mr ~26kDa.

**Species Cross-reactivity:** Human and mouse.

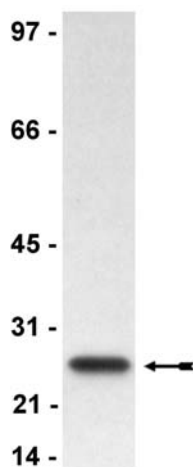
**Formulation:** 100µg protein G purified mouse IgG<sub>1</sub> in 50µl of PBS with 0.05% sodium azide before the addition of 30% glycerol. Liquid at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

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

### Quality Control Testing

Western Immunoblot Analysis: 0.5-2µg/ml of this lot detected Bcl2 in a Raji RIPA cell lysate.



#### Immunoblot Analysis

Raji cell lysate was resolved by electro-phoresis, transferred to nitro-cellulose and probed with anti-Bcl2 (1.0µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody con-jugated to HRP and a chemi-luminescence detection system. Arrow indicates Bcl2 (~26kDa).

### Additional Research Applications

Immunohistochemistry: An independent laboratory has reported that this antibody detects Bcl2 in paraffin and acetone-fixed tissues.<sup>1,2,3</sup>

FACS Analysis: Recommended.

### Application References:

1. Pezzella, F., *et al.*, Am. J. Pathol. **137**: 225-232, 1990.
2. Pezzella, F., *et al.*, J. Cancer **65**: 87-89, 1992.
3. Pezzella, F., *et al.*, New Eng. J. Med. **329**: 690-694, 1993.

### Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 $\mu$ g/ml aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Wash the blotted nitrocellulose with PBS-0.05% Tween for 10 minutes.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-2 $\mu$ g/ml of anti-Bcl2**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:1000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).