

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse Bcl-2 in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant mouse Bcl-2
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

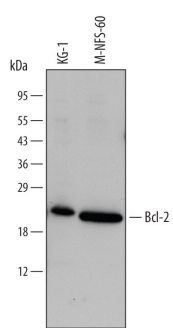
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Immunoprecipitation	1 µg/10 ⁶ cells	See Below
Simple Western	50 µg/mL	See Below

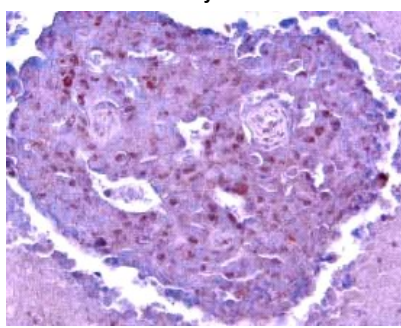
DATA

Western Blot



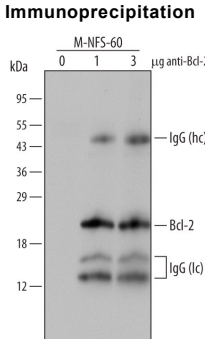
Detection of Human/Mouse Bcl-2 by Western Blot. Western blot shows lysates of KG-1 human myeloid leukemia cell line and M-NFS-60 mouse myelogenous leukemia lymphoblast cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse Bcl-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF810) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Bcl-2, at approximately 25 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunohistochemistry



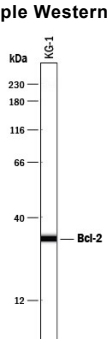
Bcl-2 in Human Breast Cancer Tissue. Bcl-2 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using 15 µg/mL Goat Anti-Human/Mouse Bcl-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF810) overnight at 4 °C. Tissue was stained (red) and counterstained with hematoxylin (blue). View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Immunoprecipitation




Immunoprecipitation of Mouse Bcl-2. Bcl-2 was immunoprecipitated from lysates (3 x 10⁶ cells) of M-NFS-60 mouse myelogenous leukemia lymphoblast cell line following incubation with 3 µg Goat Anti-Human/Mouse Bcl-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF810) for 1 hour at 4 °C. Bcl-2-antibody complexes were absorbed using Protein G (Sigma). Immunoprecipitated Bcl-2 was detected by Western blot using 1 µg/mL Goat Anti-Human/Mouse Bcl-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF810). View our [recommended buffer recipes for immunoprecipitation](#).

Simple Western



Detection of Human Bcl-2 by Simple Western™. Simple Western lane view shows lysates of KG-1 human acute myelogenous leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Bcl-2 at approximately 33 kDa (as indicated) using 50 µg/mL of Goat Anti-Human/Mouse Bcl-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF810) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Bcl-2 is a member of a family of proteins that regulates outer mitochondrial membrane permeability (1, 2). Bcl-2 is an anti-apoptotic member that prevents release of cytochrome c from the mitochondria intermembrane space into the cytosol. Bcl-2 is present on the outer mitochondrial membrane and is also found on other membranes in some cell types. Natural Bcl-2 contains a carboxyl-terminal mitochondria targeting sequence. Recombinant Bcl-2, missing the mitochondrial targeting sequence, maintains its ability to neutralize pro-apoptotic Bcl-2 family members. Neutralization by Bcl-2 appears to be through binding the BH3 region of pro-apoptotic Bcl-2 family members. This activity does not require the mitochondrial targeting sequence.

References:

1. Gross, A. *et al.* (1999) *Genes and Develop.* **13**:1899.
2. Kroemer, G. (1997) *Nature Med.* **3**:614.