

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human Bcl-2 in direct ELISAs and Western blots. In direct ELISAs, approximately 25-40% cross-reactivity with recombinant mouse (rm) Bcl-2 is observed and no cross-reactivity with recombinant human BCL2L12 is observed. In Western blots, 100% cross-reactivity with rmBcl-2 is observed.
Source	Monoclonal Mouse IgG _{2B} Clone # 625509
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Bcl-2 Ala2-Asp211 Accession # P10415
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 either lyophilized or as a 0.2 µm filtered solution in PBS.

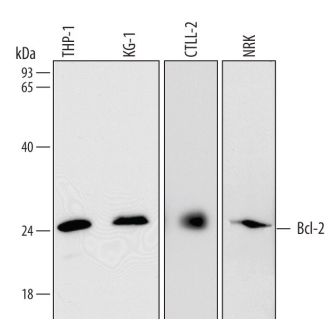
APPLICATIONS

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	0.1 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Knockout Validated	Bcl-2 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Bcl-2 knockout HeLa cell line.	

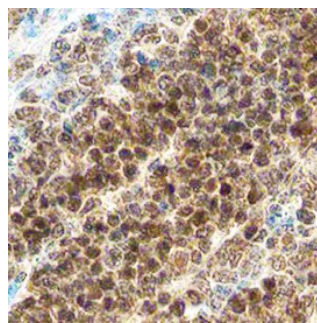
DATA

Western Blot



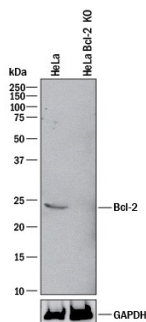
Detection of Human, Mouse, and Rat Bcl-2 by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line, KG-1 human acute myelogenous leukemia cell line, CTLL-2 mouse cytotoxic T cell line, and NRK rat normal kidney cell line. PVDF Membrane was probed with 0.1 µg/mL of Mouse Anti-Human/Mouse/Rat Bcl-2 Monoclonal Antibody (Catalog # MAB8272) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Bcl-2 at approximately 24 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

Immunohistochemistry



Bcl-2 in Human Lymphoma. Bcl-2 was detected in immersion fixed paraffin-embedded sections of human lymphoma using Mouse Anti-Human/Mouse/Rat Bcl-2 Monoclonal Antibody (Catalog # MAB8272) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and nuclei. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Knockout Validated



Western Blot Shows Human Bcl-2 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and Bcl-2 knockout HeLa cell line (KO). PVDF membrane was probed with 0.1 µg/mL of Mouse Anti-Human/Mouse/Rat Bcl-2 Monoclonal Antibody (Catalog # MAB8272) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Bcl-2 at approximately 24 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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.