Technical Data Sheet

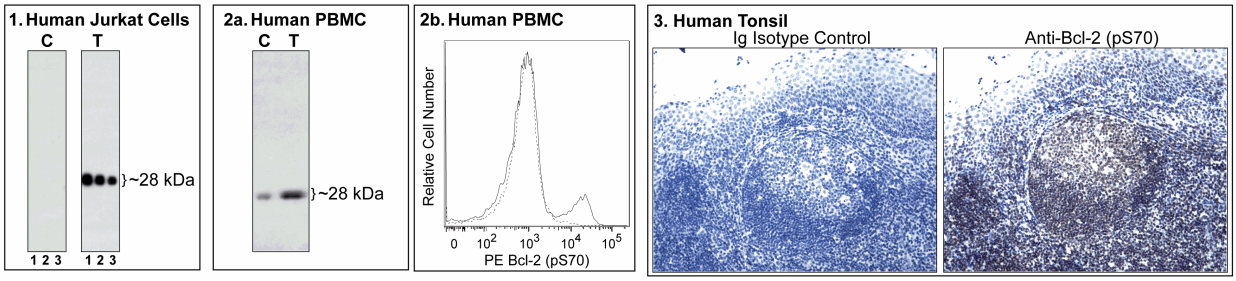
Purified Mouse anti-Human Bcl-2 (pS70)

Product Information

Material Number:	**562529**
Alternate Name:	BCL2; Apoptosis regulator Bcl-2; B-cell CLL/lymphoma 2; PPP1R50
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	N46-467
Immunogen:	Phosphorylated Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	~28 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The clone N46-467 monoclonal antibody specifically binds to Bcl-2 (pS70), ie, the Bcl-2 protein phosphorylated at the Ser70 site. Bcl-2 is a ~ 26 kDa intracellular, integral membrane protein found primarily in the nuclear envelope, endoplasmic reticulum and outer mitochondrial membrane. Bcl-2 is encoded by the *BCL2* (B-cell CLL/lymphoma 2) gene and is also known as Apoptosis regulator Bcl-2. Members of the Bcl-2 family play a major role in regulating the response of cells to apoptotic signals. Bcl-2 is one of the anti-apoptotic members of the Bcl-2 family. Bcl-2 knockout mice showed pronounced lymphoid apoptosis and other apoptosis related lesions later in life. Bcl-2 is a proto-oncogene because it blocks apoptosis and provides a selective survival advantage in many cell types and thus contributes to tumorigenesis. It has been implicated in several types of cancers, such as breast, prostate, and melanoma . Bcl-2 contains multiple phosphorylation sites including Thr56, Ser70, Thr74 and Ser87. Phosphorylation of Bcl-2 Ser70 has been shown to be a mitotic marker. Phosphorylation at this site regulates Bcl-2's anti-apoptotic activity and has recently been implicated in promoting autophagy. Several studies have shown that Bcl-2 phosphorylation is caused by c-Jun N-terminal kinase (JNK).



Analyses of Bcl-2 (pS70) expression.

Panel 1: *Western blot analysis of Bcl-2 (pS70) expressed by human Jurkat cells. Lysates (15 µg total cell protein/lane) from untreated (C) and Taxol-treated (T) (Paclitaxel, Sigma, Cat. No. T7191; 100 nM, 24 h) Jurkat cells were blotted using Purified Mouse Anti-Bcl-2 (pS70) antibody (Cat. No. 562529; 0.125, 0.063, and 0.032 µg/ml for Lanes 1, 2, and 3, respectively), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system.*

Panel 2a: *Western blot analysis of Bcl-2 (pS70) expressed by human peripheral blood mononuclear cells (PBMC). Phytohemagglutinin-stimulated (PHA, 20 µg/ml for 3 days; Sigma Cat. No. L1668) PBMC were cultured with or without Taxol (100 nM, 24 hr, 37°C). Lysates from 1 million untreated (C) and Taxol-treated (T) PBMC were blotted using Purified Mouse Anti-Bcl-2 (pS70) antibody (2.0 µg/ml) as described above.*

Panel 2b: *Flow cytometric analysis of Bcl-2 (pS70) expressed by human PBMC. PHA-stimulated (20 µg/ml for 3 days) PBMC were cultured with (solid line histogram) or without (dashed line histogram) Taxol (100 nM, 24 hr, 37°C). Cells were fixed in BD Phosflow™ Cytofix Buffer (Cat. No. 554655; 10 min, 37˚C) and permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050; 30 min, on ice) prior to staining with BD Phosflow™ PE Mouse Anti-Bcl-2 (pS70) (Cat. No. 562532) antibody. Fluorescence histograms showing Bcl-2 (pS70) expression were generated for gated events with the forward and side-light scatter characteristics of intact lymphocytes.*

Panel 3: *Immunohistochemical staining for Bcl-2 (pS70). A citrate-pretreated, formalin-fixed, paraffin-embedded tissue section of human tonsil was stained with isotype control (Cat. No. 550878; Left) or Purified Mouse Anti-Bcl-2 (pS70) antibody (Right) (20X original magnification).*

Note: *Bcl-2 (pS70) was identified as a ~28 kDa band in the cell lysates by Western blotting.*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development

The purified or conjugated mAb was characterized by flow cytometry (Flow), Western blot (WB), and immunohistochemistry (IHC) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PHA-stimulated PBMC	Nocodazole	Cytofix	Perm III	Induced in a subpopulation of cells
	Human	PHA-stimulated PBMC	Taxol	Cytofix	Perm III	Induced in a subpopulation of cells
	Human	Jurkat (serum-starved)	Taxol	Cytofix	Perm III	Induced in most cells. Blocked by pS70 phospho peptide but not by non-phospho peptide.
	Human	PBMC	PMA	Cytofix	Perm III	Weakly induced
WB	Human	PHA-stimulated PBMC	Nocodazole			28-kDa band increased
	Human	PHA-stimulated PBMC	Taxol			28-kDa band increased
	Human	Jurkat (serum-starved)	Taxol			28-kDa band increased. Blocked by pS70 phospho peptide but not by non-phospho peptide.
	Human	PBMC	PMA			28-kDa band increased
IHC	Human	Tonsil	Formalin fixed human paraffin tonsil sections with citrate buffer pretreatment			Cytoplasmic and nuclear staining observed

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
562532	PE Mouse anti-Human Bcl-2 (pS70)	50 tests	N46-467
550878	Purified Mouse IgG1 κ Isotype Control	1.0 ml	MOPC-31C

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

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