Technical Data Sheet

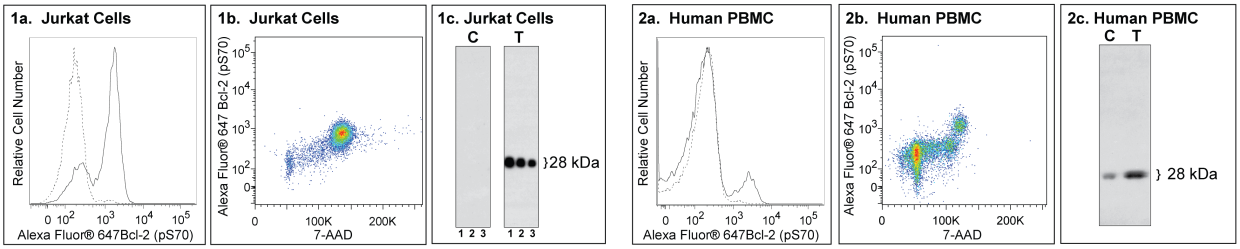
Alexa Fluor® 647 Mouse anti-Human Bcl-2 (pS70)

Product Information

Material Number:	**562531**
Alternate Name:	BCL2; Apoptosis regulator Bcl-2; B-cell CLL/lymphoma 2; PPP1R50
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N46-467
Immunogen:	Phosphorylated Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 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).



Flow cytometric (Panels 1a and 1b) and Western blot (Panel 1c) analyses of Bcl-2 (pS70) expressed by Jurkat cells. *Jurkat cells were either not treated (Panel 1a, dashed line histogram; Panel 1c, C) or treated (Panel 1a, solid line histogram; Panel 1b; Panel 1c, T) with 100 nM Taxol (Sigma, Cat. No. T7191; 24 hr, 37°C). For Panel 1a, 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 Alexa Fluor® 647 Mouse Anti-Bcl-2 (pS70) antibody (Cat. No. 562531). For optimal co-staining of total cellular DNA (Panel 1b), cells were fixed and permeabilized in 70% ethanol (30 min, on ice) prior to staining with 7-AAD (Cat. No. 559925) and Alexa Fluor® 647 Mouse Anti-Bcl-2 (pS70). Fluorescence histograms (Panel 1a) and a two-color dot plot showing DNA (7-AAD) versus Bcl-2 (pS70) levels (Panel 1b) were generated from gated events with the light scatter characteristics of intact cells using a BD™ LSR II Flow Cytometer System. For Western blot analysis (Panel 1c), cell lysates (15 µg total cell protein/lane) were blotted using Purified Mouse Anti-Bcl-2 (pS70) (Cat. No. 562529; 0.125, 0.063, and 0.032 µg/ml for Lanes 1, 2, 3, respectively). Bcl-2 (pS70) was identified as a ~28 kDa band.*

Flow cytometric (Panels 2a and 2b) and Western blot (Panel 2c) analyses of Bcl-2 (pS70) expressed by human peripheral blood mononuclear cells. *PHA-stimulated (20 µg/ml for 3 days; Sigma Cat. No. L1668) PBMC were either not treated (Panel 2a, dashed line histogram; Panel 2c, C) or treated (Panel 2a, solid line histogram; Panel 2b; Panel 2c, T) with Taxol (100 nM, 24 hr, 37°C). Flow cytometric analyses of Bcl-2 (pS70) expression without (Panel 2a) or with (Panel 2b) co-staining for DNA content were performed using a BD FACSCanto™ II Flow Cytometer System. Lysates from 1 million PBMC were blotted using Purified Mouse Anti-Bcl-2 (pS70) antibody (2.0 µg/ml) as described*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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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
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
562529	Purified Mouse anti-Human Bcl-2 (pS70)	0.1 mg	N46-467
559925	7-AAD	2.0 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
562532	PE Mouse anti-Human Bcl-2 (pS70)	50 tests	N46-467

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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