

**Bcl-2**

**Prediluted Mouse Monoclonal Antibody**

Control Number: 901-003IP-091312

**Catalog Number:** IP 003 G10  
**Description:** 10 ml, predilute

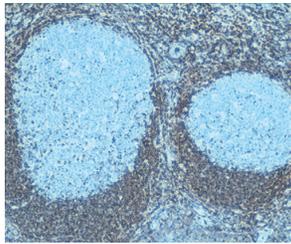
**Intended Use:**

For In Vitro Diagnostic Use

Bcl-2 [100/D5] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of Bcl-2 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

**Summary and Explanation:**

Biocare's 100/D5 MAb is highly specific to bcl-2 (alpha) and shows no cross-reaction with bcl-x or bax protein. Bcl-2 (b-cell lymphoma #2) is a proto-oncogene located at 18q21.3. Expression of bcl-2 alpha oncoprotein has been shown to inhibit programmed cell death (apoptosis). In most follicular lymphomas, neoplastic germinal centers express high levels of bcl-2 protein, whereas the normal or hyperplastic germinal centers are negative.



Tonsil stained with bcl-2a antibody.

**Principle of Procedure:**

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** 100/D5

**Isotype:** IgG1/kappa

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration.

**Epitope/Antigen:** bcl-2a

**Cellular Localization:** Cytoplasmic and nuclear membrane

**Positive Control:** Follicular lymphomas or tonsil

**Known Applications:**

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

**Storage and Stability:**

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

**Protocol Recommendations:**

**Pretreatment Solution (recommended):** Borg or Reveal

**Pretreatment Protocol:**

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

**Peroxide Block:** Block for 5 minutes at RT.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT.

**Primary Antibody:** Incubate for 30-45 minutes at RT.

**Secondary:** Incubate for 10 minutes at RT.

**Tertiary:** Incubate for 10-20 minutes at RT.

**Chromogen:** Incubate for 5 minutes with DAB at RT.

**Counterstain:**

1. Rinse with deionized water.
2. Incubate for 5 minutes with automated hematoxylin.
3. Rinse with TBS Buffer for 1 minute followed by a rinse with deionized water.

**Staining Procedure:**

Pre-optimized intelliPATH protocols with preset parameters can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the Operator's Manual for additional instruction to navigate intelliPATH software and stainer. Use TBS for washing steps unless otherwise specified.

**Limitations:**

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

**Quality Control:**

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

**Precautions:**

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The MSDS is available upon request and is located at <http://biocare.net/support/msds/>.



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**Troubleshooting:**

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

**Warranty:**

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

**References:**

1. Alderson LM, *et al.* Human gliomas with wild-type p53 express bcl-2. *Cancer Research*. 1995 Mar 1, 55(5):999-1001.
2. Hurlimann J, *et al.* bcl-2 protein in invasive ductal breast carcinomas. *Virchows Archiv*. 1995, 426(2):163-8.
3. Symmans WF, *et al.* Transformation of follicular lymphoma. Expression of p53 and bcl-2 oncoprotein, apoptosis and cell proliferation. *Acta Cytologica*. 1995 Jul-Aug, 39 (4):673-82.
4. Triscott JA, Ritter JH, Swanson PE, Wick MR. Immunoreactivity for bcl-2 protein in cutaneous lymphomas and lymphoid hyperplasias. *Journal of Cutaneous Pathology*. 1995 Feb, 22(1):2-10.
5. Bhargava V, Kell DL, van de Rijn M, Warnke RA. Bcl-2 immunoreactivity in breast carcinoma correlates with hormone receptor positivity. *American Journal of Pathology*. 1994 Sep, 145(3):535-40.
6. Joensuu H, Pylkkanen L, Toikkanen S. Bcl-2 protein expression and long-term survival in breast cancer. *Am J of Pathology*. 1994, 145(5):1191-8.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA 2005.

