

## Bcl-2

Concentrated and Prediluted Monoclonal Antibody  
901-003-031519

**BIOCARE**  
M E D I C A L

<b>Catalog Number:</b>	<b>CM 003 A, C</b>	<b>PM 003 AA</b>	<b>IP 003 G10</b>	<b>OAI 003 T60</b>	<b>VLTM 003 G20</b>
<b>Description:</b>	0.1, 1.0 mL, conc.	6.0 mL, RTU	10 mL, RTU	60 tests, RTU	20 mL, RTU
<b>Dilution:</b>	1:100	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use
<b>Diluent:</b>	Da Vinci Green	N.A	N/A	N/A	N/A

### 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:

Bcl-2 [100/D5] mouse antibody 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 the 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.

### 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 one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** 100/D5

**Isotype:** IgG1/kappa

**Protein Concentration:** 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM003 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

### Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block (Optional):** Incubate for 10-20 minutes with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

### Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Borg or Reveal Decloaker. Refer to the Borg or Reveal Decloaker product data sheet for specific instructions.

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

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

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Warp Red.

### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### intelliPATH FLX Automated Slide Stainer:

IP003 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

### Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI003 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** Bcl-2

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Option):** DS Buffer

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Reagent Name, Time, Temp.:** Bcl-2, 30 min., 25°C

### 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

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### Limitations Cont'd:

products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

### 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) (7)
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. (8)
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 SDS is available upon request and is located at <http://biocare.net>.

### 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.

### 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, *et al.* Immunoreactivity for bcl-2 protein in cutaneous lymphomas and lymphoid hyperplasias. *Journal of Cutaneous Pathology*. 1995 Feb, 22(1):2-10.
5. Bhargava V, *et al.* 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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.