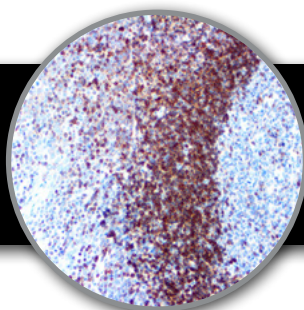


bcl-2**Clone: BSB-5****Mouse Monoclonal**
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Inset: IHC of bcl-2 on a FFPE Tonsil Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

A synthetic peptide corresponding to residues in the N-terminus of human bcl2.

Summary and Explanation

bcl-2 is an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes. Constitutive expression of bcl-2, such as in the case of translocation of bcl-2 to Ig heavychain loci, is thought to be the cause of Follicular Lymphoma.

Anti-bcl-2 has shown consistent negative reaction on reactive germinal centers and positive staining of neoplastic follicles in Follicular Lymphoma. Consequently, this antibody is valuable when distinguishing between reactive and neoplastic follicular proliferation in lymph node biopsies. This antibody may also be used in distinguishing between those Follicular Lymphomas that express bcl-2 protein and the small number in which the neoplastic cells are bcl-2-negative. Anti-bcl-2 has been used as a predictive biomarker for recurrence of Cancer of the Breast and Non-Small-Cell Carcinoma of the Lung.

Antibody Type	Mouse Monoclonal	Clone	BSB-5 (BCL2/A4)
Isotype	IgG1/K	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Tonsil, Lymph Node
Species Reactivity	Human		

Presentation

Anti-bcl-2 is a mouse monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Presentations

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 5071	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 5072	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 5073	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 5074	Concentrated	1:50 - 1:200	0.1 mL
BSB 5075	Concentrated	1:50 - 1:200	0.5 mL
BSB 5076	Concentrated	1:50 - 1:200	1.0 mL
BSB 5077	Control Slides	Not Applicable	5 slides

Precautions

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN₃), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.
3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.
6. For complete recommendations for handling biological specimens please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (8).

Storage

Store at 2-8 °C. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation to ensure best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a Steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with IHC wash buffer or DI water.
9. Continue IHC staining protocol.

Recommended IHC Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain	Varies	Varies	Varies

Performance Characteristics

Normal Tissues

Positive (+)	
Tonsil	10/10 (100%)
Lymph Node	15/15 (100%)
Negative (-)	
Liver	0/10 (0%)
Lung	0/10 (0%)
Kidney	0/10 (0%)

Abnormal Tissues

Positive (+)	
Follicular Lymphoma	5/6 (83%)
Hairy Cell Leukemia	4/5 (80%)
Negative (-)	
Anaplastic Lymphoma	0/4 (0%)

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a medical professional.

References

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3. Pezzella F, et al. *Am J Pathol*. 1990;137:225-232
4. Hockenbery D, et al. *Nature*. 1990;348:334-336
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6. Ciocca DR, Elledge R, *Endocrine*. 2000;Aug;13(1):1-10
7. Martin B, et al. *Br J Cancer*. 2003 Jul7;89(1):55-64
8. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. *Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories*. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

<div>EC REP</div> <div>EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands</div>	<div></div> <div>Storage Temperature Limites de température Zulässiger Temperaturbereich</div>	<div></div> <div>Manufacturer Fabricant Hersteller</div>	<div>REF</div> <div>Catalog Number Référence du catalogue Bestellnummer</div>
<div>IVD</div> <div>In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum</div>	<div></div> <div>Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten</div>	<div></div> <div>Expiration Date Utiliser jusque Verwendbar bis</div>	<div>LOT</div> <div>Lot Number Code du lot Chargenbezeichnung</div>



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