P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.ScyTek.com

Bcl-2; Clone 100/D5

Catalog Number	Format	Volume
A00119-0002	(Ready-To-Use)	2 ml
A00119-0007	(Ready-To-Use)	7 ml
A00119-0025	(Ready-To-Use)	25 ml
A00119-C.1	(Concentrate)	0.1 ml
A00119-C	(Concentrate)	1 ml

Intended Use

For In-Vitro Diagnostic Use. This antibody is intended for the qualitative visualization of the anatomical elements listed in the Specificity section. It is intended to be used within an Immunohistochemistry (IHC) procedure on formalin-fixed paraffin-embedded (FFPE) human tissue followed by visualization by light microscopy.

Description

Titer/Working Dilution: Ready-to-Use: No further dilution required.
Concentrate: Immunohistochemistry: 1:125 – 1:250
Western Blot Analysis: 1:300 – 1:1000

Species: Mouse

Immunogen: A synthetic peptide corresponding to amino acids 41-54 (GAAPAPGIFSSQPG-Cys), of human Bcl-2 was used as immunogen to generate the antibody; GenBank no. NP_000648.2 (Pezzella et al, 1990). This sequence is 100% conserved in the human alpha (239 aa) and beta (205 aa) isoforms. Note: this antibody clone is designated as 100 in the Pezzella publication.

Clone: 100/D5

Isotype: Mouse IgG1, Kappa

Format: Ready-To-Use antibody has been pre-titered and quality controlled to work on formalin-fixed paraffin-embedded as well as acetone fixed cryostat tissue sections. No further titration is required.
Concentrate antibody is provided in a phosphate buffered saline containing 1% BSA.

Specificity: Human Bcl-2 alpha is a 239 amino acid (aa) protein and human Bcl-2 beta is a 205 aa protein. The 100/D5 antibody [also known as clone Bcl-2/100 (Kren, 2004, Kaur, 2004)] recognizes both Bcl-2 isoforms (Pezzela et al, 1990).

Background: Bcl-2 (Bcl2) is the founding member of the Bcl-2 family. Family members have pivotal roles in regulating apoptosis or death signaling pathways through their control of mitochondrial permeability and cytochrome release (reviewed in Anvekar, 2011; Martinou and Youle, 2011). Bcl-2 derives its name from B cell lymphoma 2 where was first found to be highly expressed in follicular lymphomas with 14;18 reciprocal translocations. There are two isoforms, alpha and beta, generated by alternative splicing and differing in their carboxy termini. Human Bcl-2 alpha is a 239 amino acid (aa) protein and human Bcl-2 beta is a 205 aa protein. The 100/D5 antibody [also known as clone Bcl-2/100 (Kren, 2004, Kaur, 2004)] recognizes both Bcl-2 isoforms (Pezzela et al, 1990).

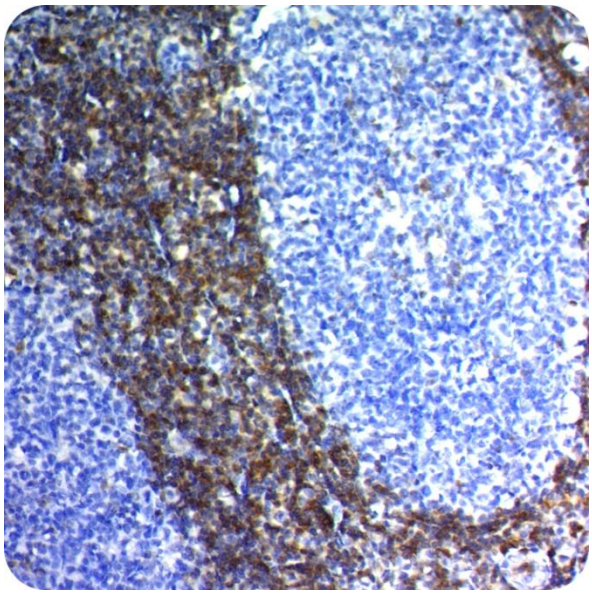
Bcl-2 is over expressed in neoplastic germinal centers of a majority of follicular lymphomas, whereas the normal or hyperplastic germinal centers are primarily negative for Bcl-2 expression. Upregulation has also been described in a number of other types of tumors. Bcl-2 expression is often considered to be a marker of cell death status, and over or high expression has often been tied to anti-apoptotic states, or resistance to death. However, the actual status of vulnerability to death can depend on the balance of other Bcl-2 family members present, their interaction with one another, as well as other factors.

Species Reactivity: Human, Monkey. Others not tested.
Positive Control: Tonsil.
Cellular Localization: Cytoplasmic & Cell Membrane.
Microbiological State: Nonsterile

Materials and Reagents Required but not Provided

1. Control tissue and reagents
2. Xylene, graded alcohols, and deionized/distilled water
3. Antibody Diluent.
4. IHC detection system. Suggested: ScyTek Cat# ABZ125 “CRF Anti-Polyvalent HRP Polymer” and ScyTek Cat# ACV500 “DAB Chromogen/Substrate Kit (High Contrast)”.
5. Wash buffer for rinses (ScyTek Cat# TBT500)
6. HIER Retrieval Solution
7. Hematoxylin counterstain and bluing reagent (ScyTek Cat# HMM500 and BRT500)
8. Mounting medium and coverslips

Note: ScyTek Laboratories has a wide range of IHC reagents and ancillaries that can be found at scytek.com.



FFPE Human Tonsil Stained with Bcl-2; Clone 100/D5. 200X

Storage: 2° C – 8° C

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Procedure

1. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed paraffin embedded tissue sections is significantly enhanced by pretreatment with Tis-EDTA HIER Solution (10x) pH 9.0 (ScyTek catalog# TES500) or Citrate Plus (10x) HIER Solution (ScyTek catalog# CPL500)

2. **Primary Antibody Incubation Time:** We suggest an incubation period of 30 minutes at room temperature. However, depending upon the fixation conditions and the staining system employed, optimal incubation should be determined by the user.

3. **Visualization:** For maximum staining intensity we recommend the "CRF Anti-Polyvalent HRP Polymer" (ScyTek catalog# ABZ125, see IFU for instructions) combined with the "DAB Chromogen/Substrate Bulk Pack (High Contrast)" (ScyTek catalog# ACV500, see IFU for instructions).

Storage and Stability

Do not Freeze. Store at 2-8°C. Return to 2-8° immediately after use. Do not use after expiration date printed on label. Verify visually that antibody has not been contaminated before use. Do not use if reagent becomes cloudy or precipitates.

Limitations

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue specimen may cause variations in results. Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. This data sheet's recommendations and procedures were validated using ScyTek IHC reagents and may not be suitable for other detection systems.

Precautions

1. Contains Sodium Azide as a preservative (0.09% w/v), do not ingest. 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. This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.
2. Do not pipette by mouth.
3. Avoid contact of reagents and specimens with skin and mucous membranes.
4. Avoid microbial contamination of reagents or increased nonspecific staining may occur.
5. The user must validate any procedures and recommendations that differ from this data sheet.
6. The SDS may be found at scytek.com

References

1. Pezzella et al. AJP 137:225-232 (1990). WB: Fig 1 (normal human spleen), the Bcl-2 alpha isoform was detected in both reducing and non-reducing conditions. IHC (frozen)/ IHC (paraffin)/ IF/ICC: Various human cell types and tissues: Figs 2,7 and Tables 1-3. See publication for specific staining details.
2. Kren et al. Appl Immunohistochem Mol Morphol. 12:44-49 (2004). IHC (paraffin): lung cancer.
3. Kaur et al. Arch Pathol Lad Med 128:39-43 (2004). IHC (paraffin): Prostate adenocarcinoma, Figs 2,3.
4. Tralongo et al. J Med Case Reports doi:10.1186/1752-1947-6-24 (2012) IHC (paraffin): follular lymphoma, results discussed. An example of a primary follicular lymphoma negative for Bcl-2 expression.
5. Moshovi et al. Pediatr Neurosurg 47:241–247 (2011). IHC (paraffin): Embryonal tumors, Table 1.
6. Martinou JC and RJ Youle. Dev Cell 21:92-101 (2011).
7. Anvekar et al. Frontiers in Oncol doi: 10.3389/fonc.2011.00034 (2011).

Warranty

No products or "Instructions For Use (IFU)" are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our IFU or website. Our warranty is limited to the actual price paid for the product. ScyTek Laboratories, Inc. is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

Storage: 2° C  8° C

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