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Rev. 1.0
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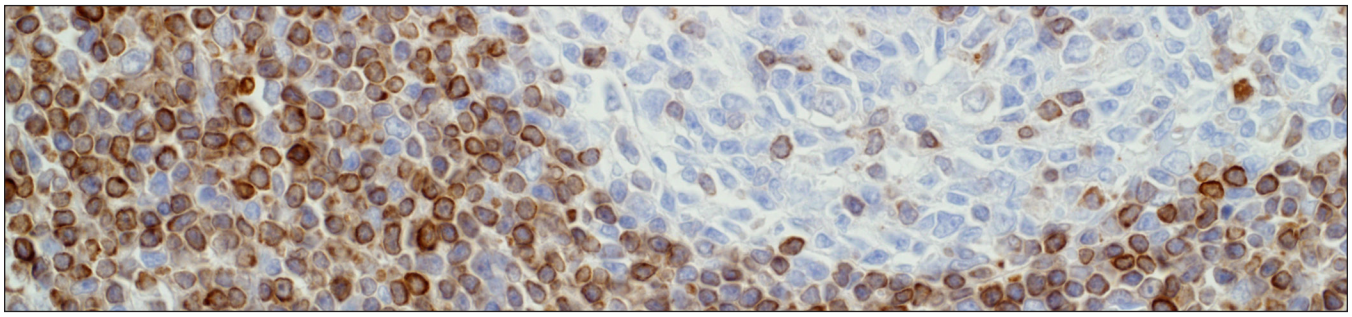
BCL2 (SP66)

Rabbit Monoclonal Antibody



PRODUCT AVAILABILITY

Cat. No.	Description
226R-24	0.1 ml, concentrate
226R-25	0.5 ml, concentrate
226R-26	1.0 ml, concentrate
226R-27	1.0 ml, Predilute ready-to-use
226R-28	7.0 ml, Predilute ready-to-use
226S	Positive control slides, 5 slides/pack

SYMBOL DEFINITIONS

P	predilute
C	concentrate
A	ascites
S	supernatant

E	serum
DIL	concentrate dilution range
KEY-CODE	keycode

INTENDED USE

This antibody is intended for *in vitro* diagnostic (IVD) use.

The Cell Marque BCL2 (SP66) antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods. Use of this antibody is indicated as an aid in distinguishing of follicular lymphoma from reactive follicular hyperplasia within the context of an antibody panel, the patient's clinical history, and other diagnostic tests evaluated by a qualified pathologist.

SUMMARY AND EXPLANATION

BCL2 is a protein associated with apoptosis regulation produced by the bcl-2 gene, located on chromosome 14q32.[1] BCL2 is comprised of an alpha (239 amino acids) and beta chain. BCL2 (and thus BCL2 alpha chain) is found in mitochondrial and nuclear membranes and in the cytosol rather than the cell surface. In normal lymphoid tissue, BCL2 (and BCL2 alpha) antibody reacts with small B-lymphocytes in the mantle zone and many cells within the T-cell areas. Anti-BCL2 alpha has shown consistent negative reaction on reactive germinal centers and positive staining of neoplastic follicles in follicular lymphoma.[2] This difference in staining pattern is not due to down regulation or decreased BCL2 mRNA, but largely to a post-translational mechanism with resultant decrease in protein levels. Consequently, this antibody is valuable when distinguishing between reactive and neoplastic follicular proliferation in lymphoid lesions. Anti-BCL2 has been used as an indicator of minimal residual disease in the bone marrow of follicular lymphoma patients when staining is strong and uniform.[3]

PRINCIPLES AND PROCEDURES

Anti-BCL2 (SP66) may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining in conjunction with a streptavidin-biotin detection system allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. Alternatively, a biotin-free polymer detection system may be used. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and cover slipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Anti-BCL2 (SP66) prediluted products are optimally diluted for use with Cell Marque detection kits though they are commonly and successfully used with a wide variety of detection kits offered by other manufacturers.

MATERIALS AND METHODS

Reagents Provided
Prediluted BCL2 (SP66) primary antibody product contains ready-to-use reagent.

Concentrated BCL2 (SP66) primary antibody product contains concentrated reagent. Both the prediluted and concentrated formats of this antibody are diluted in Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide.

The immunoglobulin concentration of both the prediluted and concentrated reagent appears on the product label.

Isotype: IgG_1

See product label for antibody source details.

Reconstitution, Mixing, Dilution, Titration
Prediluted antibody is ready-to-use and optimized for staining. No reconstitution, mixing, dilution, or titration is required. The concentrated antibody is optimized to be diluted to within the dilution range noted on the product label.

The user must validate the working dilution of the concentrated product. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and consequently require regular use of controls. (See Quality Control Procedures section)

Materials and Reagents Needed But Not Provided
The following reagents and materials may be required for staining but are not provided with the primary antibody:

1. Positive and negative control tissue
2. Microscope slides, positively charged
3. Drying oven capable of maintaining a temperature of 58-60°C ± 5°C
4. Staining jars or baths
5. Timer
6. Xylene or xylene substitute
7. Ethanol or reagent alcohol
   Note: Cell Marque's one-step pretreatment, Trilogy™ (cat. #920P-06), can replace both 6 and 7 above.
8. Deionized or distilled water
9. Electric Pressure Cooker (cat. #976L) for tissue pretreatment step
10. Detection system (such as cat. #954D-20) and chromogen (such as cat. #957D-20)
11. Wash Solutions (cat. #935B-09)
12. Hematoxylin (cat. #930B-05) or other counterstain
13. Antibody diluents (such as cat. #938B-05)
14. Peroxide Block (cat. #925B-05) for use with HRP
15. Avidin-Biotin Block (cat. # 928B-02 for use with streptavidin-biotin detection)
16. Negative Control Reagent (cat. #932B-02 for mouse; cat. #933B-02 for rabbit)
17. Mounting medium (cat. #931B-03)
18. Cover glass
19. Light microscope (40-400x)

Storage and Handling
Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the bottle must be immediately placed in the refrigerator in an upright position.

Every antibody reagent is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact Cell Marque customer service if there is a suspected indication of reagent instability.

Specimen Collection and Preparation for Analysis
Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded, tissues are suitable for use with this primary antibody when used with Cell Marque detection kits (see Materials, Reagents, and Equipment Needed But Not Provided section). Note: Cell Marque evaluates performance only on human tissues. The recommended tissue fixative is 10% neutral-buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 3 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 24 hours) in a 58-60°C ± 5°C oven.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. The user must validate incubation times and temperatures.
6. The prediluted, ready-to-use reagents are optimally diluted, and further dilution may result in loss of antigen staining.
7. The concentrated reagents may be diluted optimally based on validation by user. Any diluent used that is not specifically recommended herein must likewise be validated by the user for both its compatibility and effect on stability.
8. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% sodium azide and does not meet the OSHA (USA) criteria for hazardous substance at the stated concentration. See MSDS.
9. The user must validate any storage conditions other than those specified in the package insert.

10. Diluent may contain bovine serum albumin and supernatant may contain bovine serum. The products containing fetal bovine serum and products containing bovine serum albumin are purchased from commercial suppliers. Certificates of Origin for the animal source used in these products are on file at Cell Marque. The certificates support that the bovine sources are from countries with negligible bovine serum albumin (BSA) risk and state bovine sources as USA and/or Canada.
11. As with any product derived from biological sources, proper handling procedures should be used.

INSTRUCTIONS FOR USE

Step by Step Procedure
Recommended Staining Protocols for BCL2 (SP66)

HiDef Detection™ System :

1. Deparaffinize, rehydrate, and epitope retrieve; the preferred method is the use of Heat Induced Epitope Retrieval (HIER) techniques using Cell Marque's Trilogy™ in conjunction with a pressure cooker. The preferred method allows for simultaneous deparaffinization, rehydration, and epitope retrieval. Upon completion, rinse with 5 changes of distilled or deionized water.
2. If using HRP detection system, place slides in peroxide block for 10 minutes; rinse. If using AP detection system, omit this step.
3. Apply the antibody and incubate for 10 - 30 minutes; rinse.
4. Apply the HiDef Detection™ Amplifier Rabbit/Mouse for 10 minutes; rinse.
5. Apply the polymer detector for 10 minutes; rinse.
6. Apply ample amount of chromogen and incubate for 1 - 10 minutes; rinse.
7. Dehydrate and coverslip.

QUALITY CONTROL PROCEDURES

Positive Tissue Control
A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Use of a tissue section fixed or processed differently from the test specimen will serve to provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. Positive tissue control for BCL2 (SP66) primary antibody may include the following:

Tonsil	Cytoplasmic

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Negative Tissue Control
The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Unexplained Discrepancies
Unexplained discrepancies in controls should be referred to Cell Marque Customer Service immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negative Control Reagent
A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration. The incubation period for the negative control reagent should equal the primary antibody incubation period.

INTERPRETATION OF RESULTS

The immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody. Refer to the appropriate detection system package insert for expected color reactions. A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting results.

Positive Tissue Control
The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection system used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. If the positive tissue control fails to demonstrate appropriate positive staining, any results with the test specimens are considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from tissues that are not optimally fixed. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells show non-specific staining.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false negative reactions (see Summary of Expected Results section). The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

1. This reagent is "for professional use only" as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
2. For laboratory use only.
3. For *in vitro* diagnostic use.
4. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the tissue.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies if applicable. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
7. Cell Marque provides antibodies and reagents at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
8. Cell Marque provides primary antibodies in concentrated format so that the user may subsequently optimally dilute for use subject to the user's determination of and adherence to suitable validation techniques. Users must validate the use of any diluents other than what is recommended herein. Once the primary is validated to be suitable for use, any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users in any circumstance must accept responsibility for interpretation of patient results.
9. This product is not intended for use in flow cytometry.
10. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Cell Marque customer service with any suspected, documented unexpected reactions.
11. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
12. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
13. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) subject to the type of immunostaining technique used.
14. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

Specific Limitations

1. The prediluted antibody products are optimized as a ready-to-use product. Because of the possibility of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
2. The antibody, in combination with detection systems and accessories, detects antigen(s) that survive routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures remain, as they would in any circumstance, responsible for interpretation and validation of patient results.

Summary of Expected Results
See the following tables of reactivity:

Normal Study			
Tissue	# Stained	Total #	Notes
Brain	0	1	
Adrenal Cortex	0	1	
Ovary	1	1	Weak stain of fallopian tubal epithelial cells
Pancreas	0	1	
Parathyroid	0	1	
Pituitary	0	1	
Thyroid	0	1	
Breast	0	1	
Spleen	1	1	
Tonsil	5	5	
Thymus	0	1	
Bone Marrow	0	1	
Lung	0	1	
Heart	0	1	
Esophagus	0	1	
Stomach	0	1	
Colon	0	1	
Liver	0	1	
Kidney	0	1	
Bladder	0	1	
Prostate	0	1	
Testis	0	1	
Uterus	0	1	
Fallopian Tube	1	1	Weak stain of epithelial cells
Cervix	1	1	Weak stain of basal cells
Skeletal Muscle	0	1	
Smooth Muscle	0	1	
Skin	0	1	
Fat	0	1	
Placenta	0	1	

Disease Tissue Study			
Tissue	# Stained	Total #	Notes
Renal cell carcinoma	0	1	

Disease Tissue Study			
Hepatocellular cancer	0	1	
Melanoma	0	1	
Pancreatic carcinoma	0	1	
Lung adenocarcinoma	0	1	
Colon cancer	0	1	
Prostate cancer	0	1	
Breast cancer	0	1	
GIST	1	1	
T-cell lymphoma	0	1	
FL	6	6	
CLL/SLL	1	1	
MCL	1	1	
BL	0	1	
Follicular Hyperplasia	0	6	Negative stain of follicular center cells

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same staining run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.

2. If the positive control is negative, check other positive controls used on the same run should be checked to determine if the underlying cause relates to the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage, and fixation.

3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included unless a biotin-free detection system is being used in which case any biotin present would not be a contributing factor to background staining.

4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.

5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged. Other possibilities that could have adverse affect on tissue adhesion include insufficient drying of the tissue section on the slide prior to staining or fixation in formalin that was not properly neutral-buffered. Tissue thickness may also be a contributing factor.

For corrective action, refer to the Step by Step Procedure section or contact Cell Marque customer service.

REFERENCES

1. AS-Y Leong, K Cooper, FJW-M Leong. Manual of diagnostic antibodies for immunohistochemistry. 2nd edition. 2003 p. 25-27
2. Cooper K, Haffajee Z. Journal of Pathology 1997; 182:307-10
3. Chetty R, Echezarrata G, Comley M, et al. J Cin Pathol. 1995;48(11):1035-1038

DISCLAIMERS

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