

CONFIRM anti-bcl-2 (124) Mouse Monoclonal Primary Antibody

REF 790-4464

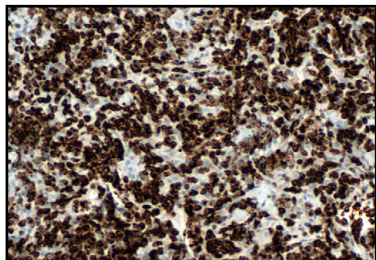


Figure 1. CONFIRM anti-bcl-2 (124) cytoplasmic staining of lymphoma tissue

INTENDED USE

Ventana Medical Systems' (Ventana) CONFIRM anti-bcl-2 (124) Mouse Monoclonal Primary Antibody (CONFIRM anti-bcl-2 (124)) is directed against human bcl-2 expressed by B cells of the mantle zone and interfollicular T cells. This antibody exhibits a cytoplasmic staining pattern and may be used to aid in the identification of follicular lymphomas and diffuse large cell lymphomas, and to differentiate follicular lymphomas from reactive lymph nodes.

CONFIRM anti-bcl-2 (124) is designed to qualitatively detect the presence of cells expressing bcl-2 protein via light microscopy in formalin-fixed, paraffin-embedded tissue.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

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

SUMMARY AND EXPLANATION

The bcl-2 oncoprotein plays a central role in apoptosis, serving as an inhibitor of the apoptotic process, and it has given name to a family of proteins engaged in the promotion/inhibition of apoptosis.¹ The expression of bcl-2 proved to block programmed cell death rather than promote proliferation. The expression of bcl-2 is inhibited in germinal centers where apoptosis forms part of the B cell developmental pathway. Reactive follicles show no staining for bcl-2, while the cells in neoplastic follicles exhibit membrane and/or cytoplasmic staining. Anti-bcl-2 has found numerous applications in studies of apoptosis, e.g., in hematological malignancies and other malignant diseases.^{2,3}

CONFIRM anti-bcl-2 (124) is a mouse monoclonal antibody produced against the bcl-2 oncoprotein.

REAGENT PROVIDED

CONFIRM anti-bcl-2 (124) contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-bcl-2 (124) contains approximately 13.1 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 1% carrier protein, and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 2.62 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-bcl-2 (124) is a monoclonal antibody produced from cell culture supernatant.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of:

- (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (*ultraView* Universal DAB Detection Kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser 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.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.⁴ Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents.
5. Consult local and/or state authorities with regard to recommended method of disposal.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT/BenchMark ULTRA instrument with *ultraView* Universal DAB Detection Kit is listed in Table 1.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-bcl-2 (124) with *ultraView* Universal DAB Detection Kit on a BenchMark XT/BenchMark ULTRA instrument

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁵

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are B cells of the mantle zone and interfollicular T cells found in tonsil.

STAINING INTERPRETATION

The cellular staining pattern for CONFIRM anti-bcl-2 (124) is cytoplasmic.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on a BenchMark XT/BenchMark ULTRA instrument in combination with *ultraView* Universal DAB Detection Kit (REF 760-500) but the user must validate results obtained with this reagent.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-bcl-2 (124) was determined by testing formalin-fixed, paraffin-embedded normal and neoplastic tissues.

For normal tissues, results are as follows: (0/3) cerebrum, (0/3) cerebellum, (0/3) adrenal gland, (0/3) ovary, (0/3) pancreas, (3/3) parathyroid, (0/3) hypophysis, (0/3) testis, (0/3) thyroid gland, (0/3) breast, (3/3) spleen, (5/6) tonsil, (2/3) thymus, (0/3) myeloid, (0/3) lung, (0/3) heart, (0/3) esophagus, (0/3) stomach, (2/3) small intestine, (3/3) colon, (0/3) liver, (0/3) salivary gland, (0/3) kidney, (2/3) prostate, (0/3) endometrium, (0/3) cervix, (0/3) skeletal muscle, (0/3) skin, (0/3) nerve, (0/3) mesothelium and lung.

For neoplastic tissues, results are as follows: (0/6) adenocarcinoma, (0/1) glioblastoma, (0/1) atypical meningioma, (0/1) malignant ependymoma, (0/1) malignant oligodendroglioma, (0/1) serous papillary adenocarcinoma, (0/1) mucinous papillary adenocarcinoma, (0/1) islet cell carcinoma, (0/1) seminoma, (0/1) embryonal carcinoma, (1/1) medullary carcinoma, (1/1) papillary carcinoma, (0/1) lobular carcinoma in situ, (0/1) invasive ductal carcinoma, (0/1) small cell undifferentiated carcinoma, (1/3) squamous cell carcinoma, (0/1) adenocarcinoma, (0/1) mucinous adenocarcinomas, (2/3) intermediate grade interstitialoma, (0/1) hepatocellular carcinoma, (0/1) hepatoblastoma, (0/2) clear cell carcinoma, (0/1) transitional cell carcinoma, (0/1) leiomyoma, (0/1) endometrial adenocarcinoma, (0/2) squamous cell carcinoma, (1/1) embryonal rhabdomyosarcoma, (1/1) malignant melanoma, (0/1) basal cell carcinoma, (0/1) neurofibroma, (1/1) neuroblastoma, (0/1) epithelial malignant mesothelioma, (3/3) diffuse malignant lymphoma, (0/1) transitional cell carcinoma with squamous metaplasia, (1/1) low grade leiomyosarcoma, (1/1) osteosarcoma, (0/1) spindle cell rhabdomyosarcoma, (0/1) intermediate grade leiomyosarcoma, (44/67) diffuse B-cell lymphoma, (1/1) diffuse small B-cell lymphoma/chronic B-cell leukemia, (1/1) nodular B-cell lymphoma, (4/5) nodular diffuse B-cell lymphoma, (1/3) B-cell mucosa-associated lymphoma, (3/3) follicular non-Hodgkin's lymphoma, (1/1) Burkitt-like lymphoma, (2/2) lymphocytic plasmacytoid lymphoma, (0/1) follicular centrocytic lymphoma, (1/1) lymphoblastic T-cell lymphoma, (1/1) diffuse T-cell lymphoma, (1/1) clear cell T-cell lymphoma, (0/1) Lennert lymphoma, (3/6) anaplastic large cell lymphoma, (0/4) mixed cellularity Hodgkin's lymphoma, (0/2) nodular sclerosis Hodgkin's lymphoma, (0/4) lymphocyte predominant type Hodgkin's lymphoma, (1/2) B-cell lymphoma, (1/2) B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, (0/2) extranodal marginal zone B-cell lymphoma, (2/7) mucosa-associated B-cell lymphoma, (1/1) peripheral T-cell lymphoma, (1/3) angioimmunoblastic T-cell lymphoma, (1/2) precursor T lymphoblastic lymphoma, (1/2) diffuse T-cell lymphoma, (0/1) T-cell lymphoma, (1/1) follicular B small cleaved cell lymphoma, (0/1) peripheral T-cell lymphoma, (0/1) MALT lymphoma, high grade, (12/22) non-Hodgkin's B-cell lymphoma, (0/2) non-Hodgkin's B-cell lymphoma, MALT, (3/3) diffuse large B-cell lymphoma, (1/2) Hodgkin's lymphoma, (1/1) reactive lymphoma, (7/8) mantle cell lymphoma.

2. Inter-lot reproducibility was determined by testing 3 lots across 1 multi-tissue block (3 tissues per block, 2 slides per lot) on a BenchMark XT instrument. 18 out of 18 tissues tested across all 3 lots scored equivalently.
3. Inter-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues each) across 5 slides on a BenchMark XT instrument over a 5 day non-consecutive period. 144 out of 150 tissue samples tested scored equivalently.
4. Intra-run repeatability was determined by staining 2 multi-tissue blocks (3 tissues each) across 14 slides on a BenchMark XT instrument. 84 out of 84 tissue samples tested scored equivalently.
5. Intra-platform repeatability was determined by staining 2 multi-tissue blocks (3 tissues each) across 5 slides on 3 BenchMark XT instruments. 90 out of 90 tissue samples tested scored equivalently.
6. Intra-platform repeatability was determined by staining 1 multi-tissue block (3 tissues) across 5 slides on 3 BenchMark ULTRA instruments. 45 out of 45 tissue samples tested scored equivalently.
7. Inter-platform repeatability was determined by staining 1 multi-tissue block (3 tissues) across 5 slides on 3 BenchMark XT instruments and 3 BenchMark ULTRA instruments. 45 out of 45 tissue samples tested scored equivalently.
8. Compatible with *VIEW* DAB and *ultraView* Universal DAB Detection Kits.

REFERENCES

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