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

REF 790-4464

05986826001

IVD Σ 50

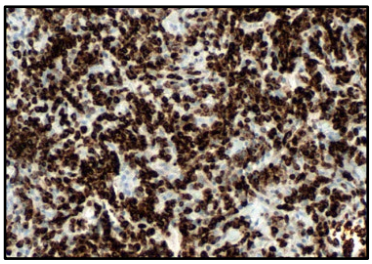

Figure 1. CONFIRM anti-bcl-2 (124) antibody cytoplasmic staining of lymphoma tissue.

INTENDED USE

CONFIRM anti-bcl-2 (124) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of B-cell lymphoma 2 protein (bcl-2) by light microscopy in sections of formalin-fixed, paraffin-embedded (FFPE) tissue stained on a BenchMark IHC/ISH instrument.

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

CONFIRM anti-bcl-2 (124) Mouse Monoclonal Primary Antibody (CONFIRM anti-bcl-2 (124) antibody) is directed against human bcl-2 protein. 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. Bcl-2 is normally expressed in T cells, pre-B cells, resting B cells including normal mantle zone lymphocytes, and certain types of proliferating B cells.[2,3] However, bcl-2 is down-regulated in normal germinal center B cells.[2,3] Among neoplastic tissues, high bcl-2 levels are detected in most human small mature B-cell lymphomas (e.g., chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma and marginal zone lymphoma), whereas it is expressed to varying degrees in diffuse large B-cell lymphoma, Hodgkin lymphoma and T-cell lymphoma.[2,4] Burkitt's lymphoma is typically bcl-2-negative, although weak expression is observed in some cases.[2,4,5] Furthermore, bcl-2 expression is also detected in many non-hematopoietic malignancies, including non-small cell lung cancer, breast cancer, colon cancer, and melanoma.[6] The mechanisms underlying bcl-2 overexpression vary widely in these neoplasms, and include chromosomal translocation, gene amplification, and microRNA dysregulation.[6]

Bcl-2 overexpression is a hallmark of follicular lymphoma; the vast majority of follicular lymphoma have the characteristic t(14;18) chromosomal translocation that puts the bcl-2 gene under the control of the IgH promoter, leading to its overexpression.[7,8] Thus, the detection of bcl-2 by immunohistochemistry (IHC) with CONFIRM anti-bcl-2 (124) antibody may be used as an aid in the identification of follicular lymphoma.

Per the 2016 revised WHO classification of lymphoid neoplasms, once the diagnosis of diffuse large B-cell lymphoma (DLBCL) is established, further characterization is required.[9] DLBCL can be characterized by cell of origin, molecular features, and genetic or mutational landscape.[10] Bcl-2 IHC is often performed in this context. Thus, the detection of bcl-2 by IHC with CONFIRM anti-bcl-2 (124) antibody may be used as an aid in the characterization of DLBCL.

Bcl-2 expression is downregulated in normal germinal center B-cells in reactive tissues, whereas it is overexpressed in the neoplastic follicles of follicular lymphoma.[7] Thus, the detection of bcl-2 by IHC with CONFIRM anti-bcl-2 (124) antibody may be used as an aid in the differentiation of follicular lymphoma from benign reactive follicles.

The staining pattern for this antibody is cytoplasmic. It may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-bcl-2 (124) antibody binds to bcl-2 protein in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001) or OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

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

One 5 mL dispenser of CONFIRM anti-bcl-2 (124) antibody contains approximately 85 µg of a mouse monoclonal antibody.

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

Specific antibody concentration is approximately 17 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-bcl-2 (124) antibody is a recombinant mouse monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. Permanent mounting medium
15. Cover glass
16. Automated coverslipper
17. General purpose laboratory equipment
18. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, 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, FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[11] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
6. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
7. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
8. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[12,13]
9. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
10. Avoid microbial contamination of reagents as it may cause incorrect results.
11. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
12. Consult local and/or state authorities with regard to recommended method of disposal.
13. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
14. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 and Table 3 below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4464.

Table 2. Recommended staining protocol for CONFIRM anti-bcl-2 (124) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

[a]Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for CONFIRM anti-bcl-2 (124) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 32 minutes	ULTRA CC1 32 minutes, 100°C
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
Pre-primary Peroxidase Inhibitor	Selected	
OptiView HQ Universal Linker	8 minutes (default)	
OptiView HRP Multimer	8 minutes (default)	
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

[a]Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[14]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-bcl-2 (124) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples.

If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

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

STAINING INTERPRETATION / EXPECTED RESULTS

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

SPECIFIC LIMITATIONS

Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised due to environmental factors during extended storage.

OptiView detection system is generally more sensitive than *ultra*View detection system. The user must validate the results obtained with this reagent and detection systems.

Up to 20% of follicular lymphoma cases containing bcl-2 translocation, do not exhibit bcl-2 IHC staining using CONFIRM anti-bcl-2 (124) antibody due to mutations on the bcl-2 gene that eliminate the epitope recognized by that antibody; however bcl-2 can be detected in those cases using antibodies that recognize alternate bcl-2 epitopes.[15,16,17]

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for specificity, sensitivity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-bcl-2 (124) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	3/3
Cerebellum	0/4	Bone marrow	2/2
Brain, NOS	0/1	Lung	0/4
Adrenal gland	0/4	Heart	0/3
Ovary	4/4	Esophagus	0/1
Pancreas	0/3	Stomach	1/4
Parathyroid gland	3/3	Small intestine	3/4
Hypophysis	3/3	Colon	3/3
Testis	0/4	Rectum	1/1
Thyroid	4/4	Liver	0/4
Breast	1/1	Salivary gland	0/4
Spleen	2/2	Kidney	4/4
Tonsil	2/2	Prostate	4/4
Reactive germinal center [a]	0/6	Bladder	4/4
Endometrium	1/1	Cervix	1/3
Myometrium	2/2	Appendix	3/3
Placenta	3/3	Skin	3/3
Skeletal muscle	3/3	Mesothelium	2/2
Peripheral nerve	0/3		

[a] Germinal center evaluated in benign tonsil and reactive lymph node.

Table 5. Sensitivity/Specificity of CONFIRM anti-bcl-2 (124) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Meningioma, fibroblastic (Brain)	1/2
Meningioma (Cerebellum)	0/1
Adenocarcinoma (Ovary)	1/1
Endometrioid adenocarcinoma (Ovary)	0/1
Colon signet ring cell carcinoma (Metastatic)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Adenoma (Thyroid)	1/1
Follicular carcinoma (Thyroid)	0/1
Follicular papillary adenocarcinoma (Thyroid)	1/1
Fibroadenoma (Breast)	2/2
Invasive ductal carcinoma (Breast)	1/3
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Cancers from gastrointestinal site (Metastatic)	0/1
Squamous cell carcinoma (Esophagus)	0/3
Adenocarcinoma (Stomach)	0/3
Adenocarcinoma (Small intestine)	0/1
Adenocarcinoma (Colon)	1/3
Adenocarcinoma (Rectum)	0/3
Adenoma (Colon)	1/1
Adenoma (Small intestine)	0/1
Hepatocellular carcinoma (Liver)	0/2
Colon adenocarcinoma (Metastatic)	0/1
Clear cell carcinoma (Kidney)	2/2
Adenocarcinoma (Prostate)	0/1
Endometrioid adenocarcinoma (Uterus)	0/2
Squamous cell carcinoma (Cervix)	0/2
Melanoma (Head and neck)	1/1
Squamous cell carcinoma (Skin)	0/1
Breast invasive ductal carcinoma (Metastatic)	0/1
Esophageal squamous cell carcinoma (Metastatic)	1/1
Chronic lymphocytic leukemia/small lymphocytic lymphoma (Lymph node)	3/3

Pathology	# positive / total cases
Diffuse large B-Cell lymphoma; NOS	131/153
Non-Hodgkin lymphoma	8/9
B-Cell follicular lymphoma	10/10
Hodgkin lymphoma (Lymph node)	1/1
Mature B-cell lymphoma; NOS (Lymph node)	1/1
Cortical adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	1/1
Adenocarcinoma (Head and neck, oral cavity, hard palate)	0/1
Adenoid cystic carcinoma (Head and neck, salivary gland)	1/1
Nasopharyngeal carcinoma (Head and neck)	0/1
Squamous cell carcinoma (Tongue)	0/1
Urothelial carcinoma (Bladder)	0/2
Osteosarcoma (Bone)	1/1

Precision

Precision studies for CONFIRM anti-bcl-2 (124) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the Intended purpose of CONFIRM anti-bcl-2 (124) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
C	Updates to Principle of the Procedure, Materials Provided, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Analytical Performance, References, and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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