

CONFIRM anti-CD20 (L26) Primary Antibody

REF 760-2531

05267099001

IVD  50

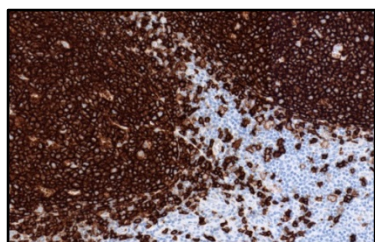


Figure 1. CONFIRM anti-CD20 (L26) Primary Antibody staining of appendix using OptiView DAB IHC Detection Kit.

automated slide stainer.

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-CD20 (L26) antibody is a mouse monoclonal antibody produced against CD20 antigen. This molecule is a non-glycosylated transmembrane phosphoprotein that is expressed on B lineage cells. Expression is first noted in the pre-B cell stage and continues throughout all stages of B-cell maturation. This antigen is not expressed on pro-B cells or plasma cells. CD20 antigen is expressed in some precursor B lymphoid neoplasms, the majority of mature B-cell neoplasms, in nodular lymphocyte predominant Hodgkin lymphoma and in a subset of classical Hodgkin lymphomas.^{2,3} CONFIRM anti-CD20 (L26) antibody can be used to detect B-cells from peripheral blood, lymph node, spleen, tonsil and bone marrow.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD20 (L26) antibody may be used as the primary antibody for immunohistochemical staining of paraffin tissue sections. CONFIRM anti-CD20 (L26) antibody binds to CD20 protein in paraffin-embedded tissue sections and exhibits a membranous staining pattern. CONFIRM anti-CD20 (L26) antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700; Mat. No. 06396500001) or *ultraView* Universal DAB Detection Kit (Cat. No. 760-500; Mat. No. 05269806001). Refer to the OptiView DAB IHC Detection Kit or *ultraView* Universal DAB Detection Kit package insert for further information.

REAGENT PROVIDED

CONFIRM anti-CD20 (L26) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD20 (L26) antibody contains approximately 1.5 µg of a mouse monoclonal (L26) antibody.

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

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 0.3 µg/mL. There is no known non-specific antibody reactivity observed for this product.

CONFIRM anti-CD20 (L26) antibody is a mouse monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (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 and ancillary components, including negative and positive tissue control slides, are not provided.

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

STORAGE

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and 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 VENTANA BenchMark IHC/ISH series automated instruments. 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 (IVD) use.
2. For professional use only.
3. 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.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark IHC/ISH series automated instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 for recommended staining protocols.

CONFIRM anti-CD20 (L26) antibody has been optimized for specific incubation and antigen retrieval times but the user must validate results obtained with this reagent. It is highly recommended that the user does not omit the cell conditioning step.

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 Protocols for CONFIRM anti-CD20 (L26) antibody with OptiView DAB IHC Detection Kit on BenchMark ULTRA, BenchMark XT, and BenchMark GX instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	BenchMark ULTRA instrument Cell Conditioning 1, 32 min BenchMark XT instrument Cell Conditioning 1, 24 min BenchMark GX instrument Cell Conditioning 1, 32 min

Procedure Type	Method
Enzyme (Protease)	None selected
Pre-primary peroxidase inhibition	Selected
Antibody (Primary)	BenchMark ULTRA instrument 16 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 6 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocols for CONFIRM anti-CD20 (L26) antibody with *ultraView* Universal DAB Detection Kit on BenchMark ULTRA, BenchMark XT, and BenchMark GX instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	BenchMark ULTRA instrument Cell Conditioning 1, Mild BenchMark XT instrument Cell Conditioning 1, Mild BenchMark GX instrument Cell Conditioning 1, Mild
Enzyme (Protease)	None selected
Antibody (Primary)	BenchMark ULTRA instrument 16 minutes, 36°C BenchMark XT instrument 16 minutes, 37°C BenchMark GX instrument 8 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 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 CONFIRM anti-CD20 (L26) antibody are spleen, tonsil, or lymph node.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD20 (L26) antibody is membranous.

SPECIFIC LIMITATIONS

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 and placed on a positively charged glass slide. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

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

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, reproducibility and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section.

Specificity

Table 3. Specificity of CONFIRM anti-CD20 (L26) antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3*
Cerebellum	0/3	Myeloid (bone marrow)	0/3*
Adrenal gland	0/3	Lung	0/3*
Ovary	0/3	Heart	0/3
Pancreas	0/3*	Esophagus	0/3*
Parathyroid gland	0/3*	Stomach	0/3*
Hypophysis	0/3*	Small intestine	0/3*
Testis	0/3	Colon	0/3*
Thyroid	0/3*	Liver	0/3*
Breast	0/2*	Salivary gland	0/3*
Spleen	5/5*	Kidney	0/3*
Tonsil	7/7*	Prostate	0/3*
Endometrium	0/3*	Cervix	0/3*
Skeletal muscle	0/3	Skin	0/3*
Nerve (sparse)	0/3	Mesothelium of lung	0/1
Bladder	0/4*	Lymph node	7/7*

* B lymphocytes staining

Sensitivity

Table 4. Sensitivity of CONFIRM anti-CD20 (L26) antibody was determined in verification by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Ovarian serous papillary adenocarcinoma	0/1
Ovarian adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/2
Thyroid medullary carcinoma	0/1
Thyroid papillary carcinoma	0/1
Breast intraductal carcinoma	0/2

Pathology	# positive / total cases
Breast invasive ductal carcinoma	0/1
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Neuroendocrine carcinoma (esophagus)	0/1
Esophageal adenocarcinoma	0/1
Gastric signet ring cell carcinoma	0/1
Gastrointestinal adenocarcinoma	0/3
GIST	0/3
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/2
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Cervical squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Rectal malignant melanoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma (skin)	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Malignant mesothelioma	0/1
Urothelial carcinoma (bladder)	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
B-cell lymphoma	129/133
T-cell lymphoma	1/55
Anaplastic large cell lymphoma	1/4
Hodgkin lymphoma	1/3

Repeatability

Repeatability studies for CONFIRM anti-CD20 (L26) antibody were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark ULTRA instrument.

- Intra-platform reproducibility on the BenchMark ULTRA instrument, BenchMark XT instrument, and BenchMark GX instrument.
- Inter-platform reproducibility between the BenchMark ULTRA instrument, BenchMark XT instrument, and BenchMark GX instrument.

All studies met their acceptance criteria.

REFERENCES

1. Mason DY, Comans-Bitter WM, Cordell JL, Verhoeven MA, van Dongen JJ. Antibody L26 recognizes an intracellular epitope on the B-cell-associated CD20 antigen. *Am J Pathol.* 1990;136(6):1215-1222.
2. Chu PG, Loera S, Huang Q, Weiss LM. Lineage determination of CD20-B-Cell neoplasms: an immunohistochemical study. *Am J Clin Pathol.* 2006;126(4):534-544.
3. Schmid C, Pan L, Diss T, Isaacson PG. Expression of B-cell antigens by Hodgkin's and Reed-Sternberg cells. *Am J Pathol.* 1991;139(4):701-707.
4. Carson F, Hladik C. *Histotechnology: A Self Instructional Text*, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
5. Roche PC, Hsi ED. *Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology*, 6th edition. (NR Rose Ed.) ASM Press, 2002.

INTELLECTUAL PROPERTY

BENCHMARK, CONFIRM, *ultraView*, OPTIVIEW, VENTANA, and the VENTANA logo are trademarks of Roche.

All other trademarks are the property of their respective owners.

© 2016 Ventana Medical Systems, Inc.

CONTACT INFORMATION



Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)



www.ventana.com



Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany