CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody

REF 760-2505

05266912001

IVD Σ 50

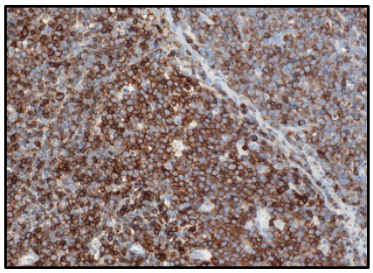

Figure 1. CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody staining of B-Cell lymphoma in tonsil.

INTENDED USE

CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of CD45 by light microscopy in sections of formalin-fixed, paraffin-embedded 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

CD45, also known as leukocyte common antigen (LCA) is a protein tyrosine phosphatase expressed on the surface of all nucleated hematopoietic cells and their precursors, except on mature erythrocytes.[1,2] CD45 is a family of five to eight glycoproteins (MW 180 to 220 kD) encoded on chromosome 1q32.[3] Various isoforms are generated by alternative splicing of three exons that can be inserted after an NH2-terminal sequence of eight amino acids found on all isoforms.[4] The various isoforms are expressed differently on different lymphoid cells and are distinguished by epitopes termed CD45RA, CD45RB, CD45RC and CD45RO.[5] CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody contains a mouse monoclonal antibody directed against the CD45RB epitope found on the membrane of leukocytic cells.[5,6] CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody has been shown to react with the 220-, 205-, and 190 kD isoforms of CD45.[5,6]

The detection of CD45 by immunohistochemistry (IHC) with the CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody may be used to detect hematolymphoid cells to aid in the diagnosis of lymphoma.

The staining pattern of this antibody is usually membranous, although cytoplasmic staining may be observed at times. It may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections. In general, immunohistochemical staining allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) that binds to the antigen, a secondary antibody (link antibody) that binds to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. 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.

CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001), and *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody contains approximately 5 µg of a mouse monoclonal antibody.

The antibody is diluted in a buffer containing carrier protein and preservative.

Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

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 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. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
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. General purpose laboratory equipment
17. 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.[7] 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. 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.
6. 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.
7. 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.[8,9]

8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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 the tables 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 760-2505.

Table 2. Recommended staining protocol for CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody with *ultra*View DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Extended	CC1, Extended	ULTRA CC1, Extended
Antibody (Primary)	8 minutes, 37°C	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-CD45, LCA (RP2/18) Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 16 minutes	CC1, 24 minutes	ULTRA CC1, 24 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	4 minutes, 37°C	4 minutes, 37°C	4 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.

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."[10]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-CD45, LCA (RP2/18) Primary 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.

Example of positive control tissue for this antibody is tonsil.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody is membranous and cytoplasmic.

SPECIFIC LIMITATIONS

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

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

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

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

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Esophagus	0/3
Cerebellum	0/3	Stomach	0/3
Adrenal gland	0/3	Small intestine	0/3
Ovary	0/3	Colon	0/3
Pancreas	0/3	Liver	0/3
Lymph Node [a]	11/11	Salivary gland	0/3
Parathyroid gland	0/3	Kidney	0/3
Pituitary gland	0/3	Prostate	0/3
Testis	0/3	Bladder	0/3
Thyroid	0/3	Endometrium	0/3
Breast	0/3	Cervix	0/3
Spleen [a]	6/6	Skeletal muscle	0/3
Tonsil [a]	9/9	Skin	0/3
Thymus	3/3	Nerve	0/3
Bone marrow	3/3	Mesothelium	0/3
Lung	0/3	Nasopharynx [b]	1/1
Heart	0/3		

[a] Tissues evaluated include normal, reactive and/or chronic inflammation.

[b] Tissue evaluated has chronic inflammation.

Table 5. Sensitivity/Specificity of CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/2
Meningioma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Endometrioid carcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal Carcinoma in situ (DCIS) (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Malignant Mixed Mesenchymal Neoplasm (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Malignant Mixed Mesenchymal Neoplasm (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Malignant Mixed Mesenchymal Neoplasm (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyosarcoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Nerve)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	0/1
Diffuse Large B-cell lymphoma (DLBCL)	113/114
B cell lymphoma, NOS	17/19
MALT B-cell lymphoma	1/1
Follicular lymphoma	2/2

Pathology	# positive / total cases
Anaplastic large cell lymphoma	11/12
Peripheral T cell lymphoma	48/49
Extranodal NK/T-cell lymphoma, nasal type	7/7
NK/T-cell lymphoma	1/1
Lymphoma, NOS	15/18
Lymphoma, null type	1/1
Hodgkin lymphoma	1/8
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Pleomorphic rhabdomyosarcoma (Peritoneum)	0/1
Embryonal rhabdomyosarcoma (Skeletal muscle)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

Precision

Precision studies for CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT, BenchMark GX, 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-CD45, LCA (RP2/18) Primary Antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

References

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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 TABLE

Rev	Updates
E	Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Materials Required but not Provided, Storage and Stability, Specimen Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Staining Interpretation / Expected Results, Specific Limitations, Analytical Performance, Clinical Performance, References, Symbols, Intellectual Property, and Contact Information sections. Added BenchMark ULTRA PLUS instrument. Removed recommended protocols for *i*VIEW DAB, AEC and Enhanced Alkaline Phosphatase Red detection kits. Removed recommended protocols for NexES IHC instrument.

INTELLECTUAL PROPERTY

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