CONFIRM anti-CD15 (MMA) Mouse Monoclonal Primary Antibody

REF 760-2504

05266904001

IVD Σ 50

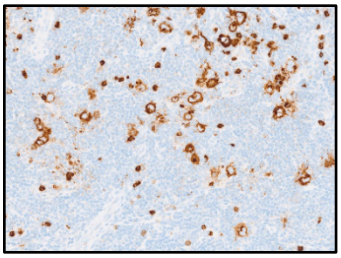

Figure 1. CONFIRM anti-CD15 (MMA) antibody staining of Hodgkin lymphoma.

INTENDED USE

CONFIRM anti-CD15 (MMA) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of CD15 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

CONFIRM anti-CD15 (MMA) Mouse Monoclonal Primary Antibody (CONFIRM anti-CD15 (MMA) antibody) recognizes lacto-N-fucopentaose III. CD15, also known as Lewis X (Lex) and stage-specific embryonic antigen-1 (SSEA-1), is a carbohydrate antigen expressed on glycolipids and glycoproteins that was first identified in mice, human milk, and some adenocarcinomas.[1,2] The antigen is formed through fucosylation, which is specifically directed by FUT4 (Fucosyltransferase 4) in promyelocytes and monocytes and FUT9 in granulocytes.[3] Of note, a related epitope, sialyl-CD15, differs in structure and modifications present on the carbohydrate molecule, and antibodies that recognize one epitope do not recognize the other.[4] Non-sialylated CD15 is involved in cell-cell adhesion and preferentially binds to P-selectin and E-selectin.[5,6] CD15 is also implicated in the activation of neutrophils and macrophages, a function that may be specific to the moiety of CD15 or selectins present.[5]

Antibodies against CD15 normally react in a strong cell surface membrane staining pattern with granulocytes and granulocyte precursors, monocytes, a subset of tissue macrophages, and activated T-lymphocytes.[4,7-11] The antigen is also expressed in activated B-cells and T-cells, follicular dendritic cells, Paneth cells, and neuroendocrine cells, and a wide range of epithelial tissues including, but not limited to, gastrointestinal tract, liver, pancreas, kidney, bladder, breast and salivary gland.[11-15]

CD15 antigen is aberrantly expressed in Hodgkin/Reed-Sternberg cells in classic Hodgkin lymphoma and, therefore, is commonly used as part of a panel to confirm a classic Hodgkin lymphoma diagnosis.[7,9,13,16]

The clinical application of the detection of CD15 by immunohistochemistry (IHC) with the CONFIRM anti-CD15 (MMA) antibody is as an aid in the diagnosis of classic Hodgkin lymphoma.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-CD15 (MMA) antibody binds to the CD15 protein in formalin-fixed, paraffin-embedded tissue (FFPE) sections and exhibits a membranous staining pattern. This antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001) or *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-CD15 (MMA) antibody contains sufficient reagent for staining 50 tests.

One 5 mL dispenser of CONFIRM anti-CD15 (MMA) antibody contains approximately 56 µg of a mouse monoclonal antibody.

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

Specific antibody concentration is approximately 11 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-CD15 (MMA) antibody is a mouse monoclonal antibody produced as a 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 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 2-8ºC. 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 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.[17] 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.

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.[18,19]
8. This product contains approximately 2% or less bovine serum which is used in the manufacture of this antibody.
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 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 760-2504.

Table 2. Recommended staining protocol for CONFIRM anti-CD15 (MMA) antibody with *ultra*View Universal DAB 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, Standard	CC1, Standard	ULTRA CC1, Standard
Antibody (Primary)	32 minutes, 37 °C	32 minutes, 37 °C	32 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-CD15 (MMA) antibody with OptiView DAB 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, 64 minutes, 95°C	CC1, 64 minutes, 100°C	ULTRA CC1, 64 minutes, 100°C
Antibody (Primary)	16 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.

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."[20]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-CD15 (MMA) 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 CONFIRM anti-CD15 (MMA) antibody are classic Hodgkin lymphoma. Hodgkin/Reed-Sternberg cell membranes should stain positively.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-CD15 (MMA) antibody is membranous as well as can demonstrate perinuclear staining with or without membrane staining.

CONFIRM anti-CD15 (MMA) antibody can also demonstrate perinuclear staining with or without membrane staining in a wide variety of cells that include but are not limited to granulocytes, neuroendocrine cells, and many types of epithelial cells.

SPECIFIC LIMITATIONS

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

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.

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

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-CD15 (MMA) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	3/3	Myeloid (bone marrow)	3/3
Cerebellum	3/3	Lymph Node	0/6
Adrenal gland	3/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	3/3	Esophagus	3/3
Parathyroid gland	0/3	Stomach	3/3
Hypophysis	2/3	Small intestine	1/3
Testis	0/3	Colon	3/3
Thyroid	0/3	Liver	0/3
Breast	2/3	Salivary gland	3/3
Spleen	0/5	Nasopharynx	0/1
Tonsil [a]	4/8	Kidney	3/3
Endometrium	3/3	Prostate	3/3
Skeletal muscle	0/3	Cervix	2/3
Soft Tissue	0/2	Skin	0/3
Nerve	0/3	Mesothelium	0/3
Thymus	3/3	Bladder	3/3
Pharynx/Oral cavity	2/3		

[a] Tissues evaluated include normal and chronic inflammation.

Table 5. Sensitivity/Specificity of CONFIRM anti-CD15 (MMA) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	1/1
CNS embryonal tumor, NOS (Cerebrum)	0/1
Serous carcinoma (Ovary)	1/1
Adult granulosa cell tumor (Ovary)	0/1
Teratoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	1/1
Ductal Adenocarcinoma (Pancreas)	1/1
Embryonal carcinoma (Testis)	1/1
Seminoma (Testis)	0/1
Follicular carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Adenoma (Adrenal Gland)	0/1
Pheochromocytoma (Adrenal Gland)	1/1
Ductal carcinoma in situ (DCIS) (Breast)	1/1
Invasive ductal carcinoma (Breast)	1/1
Invasive lobular carcinoma (Breast)	1/1
Pleomorphic adenoma (Salivary Gland)	1/1
Warthin's Tumor (Salivary Gland)	1/1
Squamous cell carcinoma (Sinus)	1/1
Adenocarcinoma (Sinus)	1/1
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	0/1
Adenocarcinoma (Esophagus)	1/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Stomach)	1/1
Gastrointestinal stromal tumor (GIST) (Stomach)	0/1
Adenocarcinoma (Small Intestine)	1/1
GIST (Small Intestine)	0/1
Adenocarcinoma (Colon)	1/1
Adenosquamous carcinoma (Colon)	1/1
Carcinoid (Appendix)	1/1
Cholangiocarcinoma (Liver)	1/1

Pathology	# positive / total cases
Hepatocellular carcinoma (Liver)	0/1
Papillary adenoma (Kidney)	1/1
Renal cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	1/1
Squamous cell carcinoma (Bladder)	1/1
Urothelial carcinoma (Bladder)	1/1
Leiomyoma (Myometrium)	0/1
Endometrioid adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	1/1
Rhabdomyosarcoma (Muscle)	0/1
Melanoma (Skin)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	1/1
Angiosarcoma (Skin)	0/1
Schwannoma (Spinal Cord)	0/1
Mesothelioma (Pleura)	0/1
Solitary fibrous tumor (Pleura)	0/1
Anaplastic large cell lymphoma	4/8
Diffuse large B cell lymphoma	13/110
Follicular lymphoma	0/2
MALT B cell lymphoma	2/9
Mantle cell lymphoma	0/1
B-cell lymphoma, NOS	4/42
Non-Hodgkin lymphoma, NOS	2/24
Peripheral T-cell lymphoma	1/3
Hodgkin lymphoma	16/19
Multiple myeloma (Bone Marrow)	0/1
Leiomyosarcoma (Abdomen)	0/1
Peripheral nerve sheath tumor (Soft Tissue)	0/1
Liposarcoma (Soft Tissue)	0/1

Precision

Precision studies for CONFIRM anti-CD15 (MMA) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, 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-CD15 (MMA) 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
F	Added the BenchMark ULTRA PLUS instruments.

INTELLECTUAL PROPERTY

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