anti-CD30 (Ber-H2) Mouse Monoclonal Primary Antibody

REF 790-4858

07007841001

IVD Σ 50

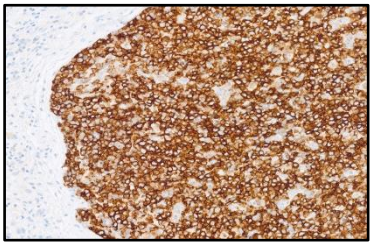

Figure 1. anti-CD30 (Ber-H2) antibody staining of anaplastic large cell lymphoma.

INTENDED USE

The anti-CD30 (Ber-H2) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of the CD30 protein 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

The anti-CD30 (Ber-H2) Mouse Monoclonal Primary Antibody (anti-CD30 (Ber-H2) antibody) is a mouse monoclonal antibody produced against a membrane bound glycoprotein that was initially described as an antigen expressed on Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma.[1,2,3] CD30 has a molecular mass of 105-120 kDa, and is a member of the tumor necrosis factor (TNF) superfamily.[4,5,6] CD30 is a costimulatory receptor normally expressed in activated, but not resting, T and B lymphocytes as well as granulocytes.[6,7,8] Its ligand, CD30L (also known as CD153) is a TNF related transmembrane protein induced on activated T-cells and antigen presenting cells.[7] In normal tissues, CD30 positive cells are seen in a small subset of cells in the parafollicular areas of tonsil and lymph nodes.[2,9,10]

Upon interacting with CD30L, CD30 is activated and forms a signaling complex with TNF receptor-associated factors.[5,11,12] CD30 mediated signal transduction is associated with the activation of pathways that influence proliferation, differentiation, and survival.[5,11] In T-cells, downstream NF-κB (nuclear factor kappa B) signaling and the activation of MAPK (mitogen-activated protein kinase) cascades induce cell proliferation and anti-apoptotic mechanisms.[5,13] Additionally, CD30 signaling induces AKT (Protein kinase B) signaling, involved in cell survival.[13] CD30 signaling in B-cells is not as well understood, but studies suggest that CD30 activation plays a role in B-cell expansion.[14]

The detection of CD30 by immunohistochemistry (IHC) with the anti-CD30 (Ber-H2) antibody may be used to aid in the diagnosis of classic Hodgkin lymphoma and anaplastic large cell lymphoma.

PRINCIPLE OF THE PROCEDURE

The anti-CD30 (Ber-H2) antibody may be used as the primary antibody for immuno-histochemical staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using the 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

Anti-CD30 (Ber-H2) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-CD30 (Ber-H2) antibody contains approximately 6 µg of a mouse monoclonal (Ber-H2) antibody.

The antibody is diluted in Tris buffered saline, EDTA, Brij-35 with carrier protein and 0.05% sodium azide, a preservative.

Specific antibody concentration is approximately 1.2 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-CD30 (Ber-H2) antibody is a recombinant mouse monoclonal antibody produced as 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. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. Amplification Kit (Cat. No. 760-080 / 05266114001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. Permanent mounting medium
16. Cover glass
17. Automated coverslipper
18. General purpose laboratory equipment
19. 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 (NBF) for a period of at least 6 hours up to 48 hours.[15] Zinc formalin fixative also is acceptable for a fixation time of at least 6 hours. The amount used is 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. A 3 mm or smaller section of tissue should be fixed no less than 4 hours and no more than 8 hours. Fixation can be performed at room temperature (15-25°C).[16]

Fixatives such as Davidson's, Modified Davidson's, B5, and other alcohol fixatives have demonstrated a loss in preservation of tissue morphology using xenograft models at the fixation times tested (12 and 72 hours), and are not recommended for use with this assay. A delay to fixation in 10% NBF greater than 2 hours also negatively impacted tissue morphology preservation.

Approximately 4 µm thick sections should be cut and picked up on positively-charged glass 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. 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.
6. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is sodium azide. Symptoms of overexposure to sodium azide include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of sodium azide in this product is 0.05% and does not meet the OSHA criteria for a hazardous substance. Buildup of sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide accumulation in plumbing.[17] Systemic allergic reactions are possible in sensitive individuals.
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. 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..

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 790-4858.

Table 1. Recommended staining protocol for anti-CD30 (Ber-H2) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes	CC1, 64 minutes	ULTRA CC1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	32 minutes, 37°C	32 minutes, 37°C	32 minutes, 36°C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 8 minutes		
Post Counterstain	Bluing, 4 minutes		

Table 2. Recommended staining protocol for anti-CD30 (Ber-H2) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	CC1, Standard	ULTRA CC1 64 minutes, 95°C
Antibody (Primary)	32 minutes, 37°C	32 minutes, 37°C	32 minutes, 36°C
Amplification	Selected	Selected	Selected (Mouse)
ultraWash	Selected	Selected	Selected
Counterstain	Hematoxylin II, 8 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."[20]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-CD30 (Ber-H2) 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. Such tissue may monitor all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen provides control for all reagents and method steps except fixation and tissue preparation.

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

Lymphoma or tonsil cases may serve as acceptable control tissues. These tissue types are suitable for optimal quality control and to detect minor levels of reagent degradation or out of specification issues which could be instrument related. In tonsil, the antibody stains scattered lymphoid cells localized around lymph follicles and at the margin of germinal centers. The positive staining tissue components (membrane, cytoplasmic, Golgi staining of neoplastic cells in lymphoma tissue or membrane, cytoplasmic, Golgi staining of lymphoid cells in tonsil) are used to confirm that the antibody was applied and the instrument functioned properly. Negative tissue components in lymphoid tissue including B cells present in the germinal centers of normal tonsil and stromal areas that may be present in lymphoma can serve to monitor background staining that may be related to the antibody, detection kit, or instrument components of the assay.

STAINING INTERPRETATION / EXPECTED RESULTS

The BenchMark IHC/ISH instrument immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by anti-CD30 (Ber-H2) antibody.

The cellular staining pattern for anti-CD30 (Ber-H2) antibody is membranous, cytoplasmic and Golgi.

Positive / Negative Tissue Controls

The stained positive and negative tissue controls should be examined to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction

product on the positive control tissue within the membrane/cytoplasm/or Golgi of the target cells is indicative of positive reactivity.

If the positive tissue control fails to demonstrate positive staining, results with the test specimens should be considered invalid.

The components that do not stain should demonstrate the absence of specific staining, and provide an indication of nonspecific background staining. If specific staining occurs in the negative tissue control sites, results with the test specimens should be considered invalid.

Negative Reagent Controls

Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in tissue sections that are excessively formalin fixed. Intact cells should be used for interpretation of staining results; as necrotic or degenerated cells often stain nonspecifically. If background staining is excessive, results from the test specimen should be considered invalid.

SPECIFIC LIMITATIONS

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.

It is recommended that patient tissue be stained within 30 days of sectioning. Loss of staining performance has been observed with the anti-CD30 (Ber-H2) antibody on sections that have been stored at room temperature for longer than 30 days.

It is recommended that sections thicker than 4 microns not be used with this assay. It is more difficult to evaluate neoplastic cell staining on tissues sectioned at ≥ 5 microns.

Weak nuclear staining in lymphocytes has been observed on very few cases stained with anti-CD30 (Ber-H2) antibody and should not be evaluated as anti-CD30 positive staining.

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 3. Sensitivity/Specificity of anti-CD30 (Ber-H2) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	0/3
Adrenal gland	0/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	0/3	Liver	0/3
Pituitary gland	0/3	Salivary gland	0/3
Testis	0/3	Kidney	0/3
Thyroid	0/3	Prostate	0/3
Breast	0/3	Endometrium	0/3
Spleen	0/3	Cervix	0/2
Tonsil	17/18	Skeletal muscle	0/2
Lymph Node	12/26	Skin	0/3
Thymus	0/3	Nerve	0/3
Bone marrow	0/3	Mesothelium	0/2
Lung	0/4		

Table 4. Sensitivity/Specificity of anti-CD30 (Ber-H2) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis) [a]	1/1
Embryonal carcinoma (Testis) [a]	1/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (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
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Intestine)	0/1
Gastrointestinal stromal tumor (Intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Abdominal cavity)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyoma (Uterus)	0/1

Pathology	# positive / total cases
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Carcinoma (Endometrium)	0/1
Clear cell carcinoma (Endometrium)	0/1
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Soft tissue)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Abdominal cavity)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Burkitt lymphoma	1/3
Follicular lymphoma	0/3
MALT lymphoma	0/1
Diffuse large B-cell lymphoma	0/12
B-cell lymphoma, NOS	1/14
Plasma cell myeloma	1/10
Hodgkin lymphoma	92/99
Anaplastic large cell lymphoma	7/9
T-cell lymphoma, NOS	2/18
Lymphoma, NOS	0/2
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Leiomyosarcoma (Smooth muscle)	0/1
Melanoma	0/1

[a] Results were consistent with expected positive/negative staining in neoplastic tissues. [21]

Precision

Precision studies for anti-CD30 (Ber-H2) 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 instruments.
- Between platform precision between BenchMark XT and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of anti-CD30 (Ber-H2) 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

INTELLECTUAL PROPERTY

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.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE