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

REF 790-4858

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IVD Σ 50

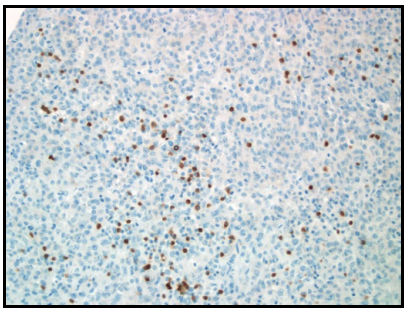

Figure 1. The anti-CD30 (Ber-H2) staining of Reed Sternberg cells in a mixed cellularity Hodgkin lymphoma.

INTENDED USE

The anti-CD30 (Ber-H2) Mouse Monoclonal Primary Antibody (anti-CD30 (Ber-H2)) is intended for laboratory use in the detection of the CD30 protein in formalin-fixed, paraffin-embedded tissue stained with a VENTANA BenchMark series immunohistochemical (IHC) automated slide stainer. CD30 positive staining results may aid in the identification of classical Hodgkin lymphoma and anaplastic large cell lymphoma.

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

This antibody is for *in vitro* diagnostic (IVD) use.

SUMMARY AND EXPLANATION

The anti-CD30 (Ber-H2) is a mouse monoclonal antibody produced against a membrane bound glycoprotein that has a molecular mass of 105-120 kD. CD30 is a member of the tumor necrosis factor superfamily which when activated results in the signaling for antiapoptotic pathways allowing for cell proliferation. CD30 antigen is expressed in mononuclear Hodgkin cells and multinucleated Reed Sternberg cells of Hodgkin lymphoma as well as on anaplastic large cell lymphomas. This antibody variably produces membranous, cytoplasmic, and Golgi staining of both lymphoma cells and of scattered large activated B cells and T cells in lymph nodes, spleen, tonsil, and thymus. This antibody may also stain a small proportion of plasma cells.[1,2,3]

PRINCIPLE OF THE PROCEDURE

The anti-CD30 (Ber-H2) may be used as the primary antibody for immunohistochemical staining of paraffin 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.

REAGENT PROVIDED

The anti-CD30 (Ber-H2) contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-CD30 (Ber-H2) contains approximately 6.15 µg of a mouse monoclonal (Ber-H2) antibody.

The antibody is diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3 % carrier protein and 0.05 % sodium azide, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 1.23 µg/mL. There is no known non-specific antibody reactivity observed in this product.

The anti-CD30 (Ber-H2) is a recombinant 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

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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA OptiView DAB IHC Detection Kit and a VENTANA BenchMark ULTRA automated slide stainer.

The recommended tissue fixative is 10% neutral buffered formalin (NBF) for a period of at least 6 hours up to 48 hours.[4] 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).[5]

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.

The Clinical Laboratory Improvement Act (CLIA) of 1988, 42CFR493.1259(b) requires that "[t]he laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination." 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. 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. Build up 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.[6] Systemic allergic reactions are possible in sensitive individuals.

3. ProClin 300 is used as a preservative in this solution. 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 Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 and Table 2 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 instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-CD30 (Ber-H2) with OptiView DAB IHC Detection Kit on a BenchMark GX instrument, BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes
Enzyme (Protease)	None required
Pre-Peroxidase Inhibitor	Selected
Antibody (Primary)	BenchMark GX instrument 32 minutes, 37°C BenchMark XT instrument 32 minutes, 37°C BenchMark ULTRA instrument 32 minutes, 36°C
OptiView HQ Linker	8 minutes
OptiView HRP Multimer	8 minutes
Counterstain	Hematoxylin II, 8 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for anti-CD30 (Ber-H2) with *ultra*View Universal DAB Detection Kit on a BenchMark GX instrument, BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark GX instrument 32 minutes, 37°C BenchMark XT instrument 32 minutes, 37°C BenchMark ULTRA instrument 32 minutes, 36°C
Amplification	Required
ultraWash	Required
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".[7]

POSITIVE TISSUE CONTROL

A control tissue, having both positive and negative elements, fixed and processed in the same manner as the patient specimens should be run for each set of test conditions and with every anti-CD30 (Ber-H2) staining procedure performed.

Control tissue should be fresh autopsy/biopsy/surgical specimens prepared and 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.

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.

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

STAINING INTERPRETATION / EXPECTED RESULTS

The VENTANA automated immunostaining procedure causes a brown colored (DAB) reaction product to precipitate at the antigen sites localized by anti-CD30 (Ber-H2). The cellular staining pattern for anti-CD30 (Ber-H2) 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 patient 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

The anti-CD30 (Ber-H2) has been optimized on VENTANA BenchMark ULTRA instruments in combination with the OptiView DAB IHC Detection Kit at a 32 minute primary antibody incubation time.

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) 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) and should not be evaluated as anti-CD30 positive staining.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, 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 anti-CD30 (Ber-H2) 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)	0/2
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	0/3	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	8/9	Prostate	0/3
Lymph Node	12/18	Cervix	0/2
Endometrium	0/3	Skin	0/3
Skeletal muscle	0/2	Mesothelium and lung	0/1
Nerve (sparse)	0/3		

Results were consistent with expected positive/negative staining for normal tissues.

Sensitivity

Table 4. Sensitivity of anti-CD30 (Ber-H2) was determined 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
Serous papillary adenocarcinoma	0/1
Mucinous papillary adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma[8]	1/1
Embryonal carcinoma[8]	1/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast intraductal carcinoma	0/1
Breast lobular carcinoma *in situ*	0/1
Breast invasive ductal carcinoma	0/2
Burkitt's lymphoma	1/3
Diffuse lymphoma	2/3
Diffuse B-cell lymphoma	0/1
Diffuse large B-cell lymphoma	8/27
Diffuse B non-cleaved cell lymphoma	0/3
Diffuse B cleaved cell lymphoma	0/1
Diffuse T-cell lymphoma	12/21
Diffuse B small cleaved cell lymphoma	0/3
Diffuse plasmacytic lymphoma	0/1
Hodgkin lymphoma	99/105

Pathology	# positive / total cases
Anaplastic large cell lymphoma	7/8
Large B-cell lymphoma	1/4
B large non-cleaved cell lymphoma	2/4
B small cleaved cell lymphoma	2/3
Plasma cell myeloma	0/4
Follicular B-cell lymphoma	0/3
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Esophageal squamous cell carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma	0/1
GIST	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/1
Prostatic transitional cell carcinoma	0/1
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	0/1
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Diffuse malignant lymphoma	0/1
Diffuse malignant lymphoma	0/1
Peripheral T-cell Lymphoma	3/3
T-cell Lymphoma	7/7
Mycosis Fungoides	7/7
Burkitt-like Lymphoma	0/1

Pathology	# positive / total cases
Sub cutaneous Paniculitis like T-cell lymphoma	1/1
Mucosa-associated lymphoma	2/2
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Malignant melanoma	0/1

Results were consistent with expected positive/negative staining for neoplastic tissues.

Repeatability

Repeatability studies for anti-CD30 (Ber-H2) were completed to demonstrate:

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

All studies met their acceptance criteria.

REFERENCES

1. Froese P, Lemke H, Gerdes J, et al. Biochemical characterization and biosynthesis of the Ki-1 antigen in Hodgkin-derived and virus-transformed human B and T lymphoid cell lines. J Immunol. 1987;139: 2081-7.
2. Stein H, Mason DY, Gerdes J, et al. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood. 1985;66:848-858.
3. Stein H, Gerdes HL, Schwab U, et al. Identification of Hodgkin and Sternberg Reed cells as a unique cell type derived from a newly-detected small cell population. Int J Cancer. 1982;30:445-59.
4. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
5. Sheehan DC, Hrapchak BB. Theory and practice of Histotechnology, 2nd edition. St. Louis: The C.V. Mosby Company; 1980.
6. Department of Health, Education and Welfare, National Institute of Occupational Safety and Health, Rockville, MD. "Procedures for the decontamination of plumbing systems containing copper and/or lead azides." DHHS (NIOSH) Publ No. 78-127, Current 13. August 16, 1976.
7. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.
8. Durkop H. Foss HD. et al. Expression of the CD30 antigen in non-lymphoid tissues and cells. J. Pathol 2000; 190:613-618. .

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