

## CD20 [L26]

Concentrated and Prediluted Monoclonal Antibody  
901-004-022219

**BIOCARE**  
M E D I C A L

Catalog Number:	CM 004 A, B, C	PM 004 AA, H	IP 004 G10, G20	OAI 004 T60	VLTM 004 G20
Description:	0.1, 0.5, 1.0 mL, conc.	6.0, 25 mL, RTU	10, 20 mL, RTU	60 tests, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Da Vinci Green	N/A	N/A	N/A	N/A

### Intended Use:

For In Vitro Diagnostic Use

CD20 [L26] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD20 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

### Summary and Explanation:

CD20 [L26] reacts with a protein of a 30-33 kDa polypeptide present in B-cells. L26 reacts with the majority of B-cells present in peripheral blood and lymphoid tissues. In normal lymphoid tissue, L26 marks B-cells in germinal centers, particularly immunoblasts. This antibody has been shown to be a reliable marker as a pan B-cell marker. It rarely marks T-cells.

### Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** L26

**Isotype:** IgG2a/kappa

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** CD20 (B-cell)

**Cellular Localization:** Cell surface

**Positive Tissue Control:** Tonsil or B-cell lymphoma

### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

### Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

### Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM004 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X – OR – Val AR-Lo pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

### Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

**Protein Block (Optional):** Incubate for 10-20 minutes with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

### Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Warp Red.

### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### intelliPATH FLX Automated Slide Stainer:

IP004 is intended for use with the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

### Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI004 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD20

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Reagent Name, Time, Temp.:** CD20, 30 min., 25°C

### Protocol Recommendations (Ventana BenchMark ULTRA):

PM004 is compatible for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 32 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 16 minutes, 36°C

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### Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

### Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA ([www.clsi.org](http://www.clsi.org)). 2011

### Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide ( $\text{NaN}_3$ ) used as a preservative is toxic if ingested. 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 build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

### Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

### References:

1. Nguyen DT, *et al.* Differential diagnosis of L26-positive, CD15-negative Hodgkin's disease and large B-cell lymphoma with a high content of reactive T-cells: a morphologic and immunohistochemical study. *Hematopathol Mol Hematol.* 1996;10(3):135-50.
2. Chadburn A, Knowles DM. Paraffin-resistant antigens detectable by antibodies L26 and polyclonal CD3 predict the B- or T-cell lineage of 95% of diffuse aggressive non-Hodgkin's lymphomas. *Am J Clin Pathol.* 1994 Sep;102(3):284-91.
3. Cartun RW, Coles FB, Pastuszak WT. Utilization of monoclonal antibody L26 in the identification and confirmation of B-cell lymphomas. A sensitive and specific marker applicable to formalin-and B5-fixed, paraffin-embedded tissues. *Am J Pathol.* 1987 Dec;129(3):415-21.
4. Norton AJ, Isaacson PG. Monoclonal antibody L26: an antibody that is reactive with normal and neoplastic B lymphocytes in routinely fixed and paraffin wax embedded tissues. *J Clin Pathol.* 1987 Dec;40(12):1405-12.
5. Davey FR, *et al.* Immuno-phenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin-embedded tissues. *Am J Pathol.* 1987 Oct;129(1):54-63.
6. Ishii Y, *et al.* Surface marker expression of human B-cell lymphomas. *AIDS Res.* 1986 Dec;2 Suppl 1:S87-93.

### References Cont'd:

7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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