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 lymphoma marker. It rarely marks T-cells.

**Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont’d:**

**Protocol 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.

**Peroxidase Block (intelliPATH FLX* and manual use):**

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

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

**Secondary 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, peroxidase block with intelliPATH FLX Peroxidase Blocking Reagent (JP85005) 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):**

OA004 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

**Peroxidase**

**DePar:** CC1 32 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** CD20

**Staining System:**

**Primary Antibody:** CD20

**Peroxidase**

**DePar:** CC1 32 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** CD20

**Staining System:**
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:

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 (NaN3) 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:

References Cont’d:

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