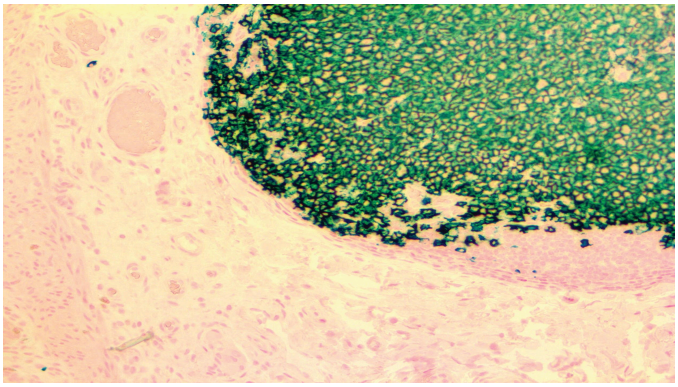
CD20 (L26)

Mouse Monoclonal Antibody

CE IVD



IHC of CD20 on FFPE Colon tissue

PRODUCT IDENTIFICATION REF

Catalog No.	Presentation	Volume
BSB 5190	Predilute Ready-to-Use	3.0 mL
BSB 5191	Predilute Ready-to-Use	7.0 mL
BSB 5192	Predilute Ready-to-Use	15.0 mL
BSB 5193	Concentrate	0.1 mL
BSB 5194	Concentrate	0.5 mL
BSB 5195	Concentrate	1.0 mL

INTENDED PURPOSE

CD20 (L26), Mouse Monoclonal Antibody is a primary antibody intended for laboratory use by trained laboratory personnel in an immunohistochemical (IHC) assay to qualitatively identify CD20 protein by light microscopy in normal and/or pathological formalin-fixed, paraffin-embedded (FFPE) human tissue.

The clinical interpretation of any staining or its absence should be performed by a qualified pathologist and complemented by morphological studies using proper controls and evaluated within the context of the patient's clinical history and other diagnostic tests.

SUMMARY AND EXPLANATION

CD20 is a transmembrane, non-glycosylated protein expressed on B-cell precursors and mature B-cells, but lost following differentiation into plasma cells. CD20 is not expressed in non-hematopoietic neoplasms, but is expressed in Reed-Sternberg cells predominant in Hodgkin's disease.

PRINCIPLE OF PROCEDURE

In general, immunohistochemical (IHC) staining techniques allow for antigen visualization via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link 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 coverslipped. Results are interpreted using a light microscope.

MATERIALS AND PRESENTATION

This monoclonal antibody is derived from cell culture supernatant and provided in a diluent at pH 7.3-7.7 containing Tris buffer, 1% BSA as a stabilizer, and <0.1% sodium azide as a preservative.

Antibody Type	Mouse Monoclonal	**Clone**	L26
Isotype	IgG2a/K	**Reactivity**	Human
Localization	Membranous	**Source**	Supernatant
Recommended Dilution Range	1:250-1:1000		
Immunogen	Human tonsil B cells.		

MATERIALS REQUIRED BUT NOT PROVIDED

Positive and negative control tissues
Positively charged microscope slides, such as Bio SB Hydrophilic Plus Slides *(BSB 7028)*
Drying oven capable of maintaining a temperature of 53-65 °C
Xylene or xylene substitute, such as Tinto Dewaxer *(BSB 7458)*
Ethanol or reagent alcohol
Distilled water
Slide handling equipment, such as staining dishes (*BSB 7009*) and slide holder *(BSB 7010)*
Heating equipment for tissue pretreatment, such as the Bio SB TintoRetriever Pressure Cooker *(BSB 7015)*
Suitable epitope retrieval solution, such as ImmunoDNA Retriever with Citrate (*BSB 0020 through BSB 0023*) or EDTA *(BSB 0030 through BSB 0033)*
IHC Wash Buffer, such as ImmunoDNA Washer *(BSB 0029, BSB 0042, BSB 0149, BSB 0150)*
Antibody diluent, such as ImmunoDetector Protein Blocker/ Antibody Diluent *(BSB 0113 through BSB 0115, BSB 0040, BSB 0041)*
Negative Control Reagent, such as *(BSB 0040A through C, BSB 0041A through C)*
Anti-Mouse detection system, such as the Bio SB PolyDetector Plus HRP Detection System (BSB 0257 through 0266)
Counterstain, such as Bio SB Hematoxylin Counterstainer *(BSB 0024 through BSB 0028)*
Mounting medium, such as PermaMounter *(BSB 0097)* or AquaMounter *(BSB 0090 through BSB 0093)*
Cover glass, such as Tinto Coverslips *(BSB-7100-100, BSB-7100-1000, BSB-7100-20000)*
Timer
Light microscope (40-400x)

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional users only. Results should be interpreted by a qualified medical professional and complemented by morphological studies using proper controls and evaluated within the context of the patient's clinical history and other diagnostic tests.
3. This product contains <0.1% sodium azide (NaN_3) as a preservative. The following hazard and precautionary statements apply: H303 - May be harmful if swallowed. P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. For additional safety information refer to the Safety Data Sheet.
4. Avoid contact with eyes. If contact occurs, flush with large quantities of water.

5. 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.
6. Materials of human and animal origin should be handled as biohazardous materials and disposed of with proper precautions. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories."
7. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. Accumulated 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.
10. Dispose of contents and container in accordance with all local, regional, national, and international regulations.
11. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).

REAGENT STORAGE AND STABILITY

1. Store at 2-8 °C in original packaging.
2. This product is stable up to the expiration date on the product label when stored according to instructions. Do not use after expiration date listed on the label.
3. Temperature fluctuations should be avoided. Directly following every run, tightly close product and place in refrigerator in an upright position. Avoid prolonged exposure to room temperature conditions.
4. There are no definitive signs to indicate instability of this product. Contact Bio SB Customer Support if there is a suspected indication of reagent instability.

PROCEDURE

Recommended Specimen Preparation

The antibody can be used on FFPE tissue sections. Ensure tissue undergoes appropriate fixation for best results.
1. Cut and mount 3-5 µm FFPE tissues on positively charged slides.
2. Air dry slides for 1 hour at 60 °C.
3. Deparaffinize and rehydrate FFPE tissues:
   - Heat slides in a 60 °C incubator for 10 min. to partially melt the paraffin.
   - Pass slides through three xylene or xylene alternative baths, 2 min. per bath
   - Pass slides through two 100% ethanol baths, 2 min. per bath
   - Pass slides through one 70% ethanol bath for 2 min.
   - Pass slides through one 30% ethanol bath for 2 min.
   - Pass slides through one distilled water bath for 2 min.
4. Subject tissues to heat-induced epitope retrieval (HIER) using a suitable HIER solution, such as Bio SB ImmunoDNA Retriever with Citrate or ImmunoDNA Retriever with EDTA. Use a heating method such as TintoRetriever Pressure Cooker or equivalent; follow the Instructions for Use for the heating method used.
5. Following retrieval, immediately remove the staining dish with slides from TintoRetriever Pressure Cooker and transfer to room temperature; let cool until the retrieval solution is no longer opaque. Wash slides with Bio SB ImmunoDNA Washer or equivalent and begin IHC protocol. Note: Tissues should remain hydrated via use of a wash buffer.

Recommended Manual Immunohistochemical Protocol

Wash slides between each step in the table below with ImmunoDNA Washer or equivalent at least 3 times, until ImmunoDNA Washer runs evenly on the slide surface.

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
HRP/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	N/A	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain/Coverslip	Varies	Varies	Varies

Recommended Automated Immunohistochemical Protocol

Perform according to the manufacturer's instructions of the applicable automated instrument.

Preparation of the Working Solution

Prediluted antibody is a ready-to-use product. The concentrated antibody should be diluted and optimized by the user.

Mounting Protocols

For instructions about using a mounting media such as PermaMounter, refer to the Instructions for Use of the product.

QUALITY CONTROL RECOMMENDATIONS

Controls should be fresh autopsy, biopsy, or surgical specimens fixed, processed, and embedded as soon as possible in the same manner as the sample(s). Such a control monitors all steps of the analysis, from tissue preparation through staining. The use of a tissue section fixed or processed differently from the test specimen will act as a control for all reagents and method steps except fixation and tissue processing.

Bio SB Control Slides Available

Catalog No.	Quantity
BSB-9078-CS	5 slides

Positive Tissue Control: A positive tissue control must be run with every test procedure. A tissue with weak positive staining (e.g., low expressor) is optimal for detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Positive tissue control for the antibody may include the following: tonsil, lymph node.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control: One tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Internal negative control sites should be verified by the user. The components that do not stain should demonstrate the absence of specific staining and provide an indication of non-specific background staining.

Negative Control Reagent: A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration.
The incubation period for the negative control reagent should equal the primary antibody incubation period.

INTERPRETATION OF RESULTS

Positive Tissue Control Interpretation: The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells/cellular components is indicative of positive reactivity. Refer to the IFU of the detection system used for expected color reactions. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

Negative Tissue Control Interpretation: The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross-reactivity to cells/cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid. Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue Interpretation: Examine stained patient specimens last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any IHC test, a negative result means that the antigen was not detected, not that the antigen was absent.

LIMITATIONS

1. Immunohistochemistry is a multi-step process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents, and methods to interpret all of the steps used to prepare and interpret the final IHC preparation.
5. Predilute Ready-to-Use antibodies are provided at optimal dilution for use following the recommended instructions for IHC on prepared tissue sections preparation. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.
6. This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.
7. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
8. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Bio SB Customer Support with documented unexpected reaction(s).
9. Normal/non-immune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
10. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), endogenous phosphatase (e.g., lymphoid, intestinal, placenta), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used.
11. Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls.

REFERENCES

1. Ishii Y, Takami T, Yuasa H, Takei T, Kikuchi K. Two distinct antigen systems in human B lymphocytes: identification of cell surface and intracellular antigens using monoclonal antibodies. *Clin Exp Immunol.* 1984;58(1):183-192.
2. Davey FR, Gatter KC, Ralfkiaer E, Pulford KA, Krissansen GW, Mason DY. Immunophenotyping of non-Hodgkin's lymphomas using a panel of antibodies on paraffin-embedded tissues. *Am J Pathol.* 1987;129(1):54-63.
3. Mason DY. A new look at lymphoma immunohistology. *Am J Pathol.* 1987;128(1):1-4.
4. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement/Vol. 61, January 6, 2012. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

SYMBOLS GLOSSARY

The following symbols may be found in this IFU or on the product labeling. Some glossary symbols may not be applicable to this product.

Source	Symbol	Meaning
ISO 15223-1 5.1.1		Manufacturer
ISO 15223-1 5.1.2		Authorized representative in the European Union
ISO 15223-1 5.1.4		Use-by-Date
ISO 15223-1 5.1.5		Batch Code (Lot Number)
ISO 15223-1 5.1.6		Catalog Number
ISO 15223-1 5.1.8		Importer
ISO 15223-1 5.3.7		Temperature Limit
ISO 15223-1 5.4.3		Consult electronic Instructions for Use
ISO 15223-1 5.4.4		Caution
ISO 15223-1 5.5.1		In Vitro Diagnostic Medical Device
ISO 15223-1 5.7.10		Unique Device Identifier
(EU) 2017/746 *In Vitro* Diagnostic Regulation (IVDR)		European Union Conformity
Bio SB Manufacturer symbol		Ready-To-Use; reagent is provided at a prediluted concentration that is ready for use
Bio SB Manufacturer symbol		Reagent is provided as a concentrate that needs to be diluted for use

CONTACT INFORMATION

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Printed IFU
Available upon request.

Note For Customers Within The European Union (EU):
Any serious incident that has occurred in relation to the device must be reported to Bio SB or local sales representative and the competent authority of the Member State in which the user and/or the patient is established.

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