

# CD3 [BC33]

Concentrated and Prediluted Monoclonal Antibody  
901-3170-032323

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

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3170 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	API 3170 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3170 T60	60 tests	Ready-to-use	N/A
Q Series – For Leica BOND-III	ALI 3170 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

CD3 [BC33] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of CD3 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:

CD3 is expressed throughout the T-cell differentiation process. CD3 is a highly specific and sensitive T-cell lineage marker, making it ideal for the immunohistochemical analysis of lymphohematologic malignancies. Notable exceptions include some of the more aggressive large T-cell lymphomas and CD30 (Ki-1) positive anaplastic large cell lymphomas, which may not express detectable antigen.<sup>1-3</sup> A monoclonal antibody to human CD3 is regarded as a reliable pan T-cell antibody used in identifying lymphomas and lymphocytic colitis in paraffin sections.<sup>4</sup>

## 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:** BC33

**Isotype:** IgG1

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

**Epitope/Antigen:** CD3

**Cellular Localization:** Cell surface

**Positive Tissue Control:** Tonsil

## 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 (intelliPATH FLX® and manual use):

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

**Pretreatment:** Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker 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.

## 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™ Pro Automated Slide Staining System):

OPAI3170 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** CD3 [BC33]

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

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** CD3 [BC33], 30 min., 25°C

## Protocol Recommendations (Q Series – For Leica BOND-III):

ALI3170 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Protocol Name:** IHC Protocol F

**Detection:** Bond Polymer Refine

**HIER:** 20 min with ER1

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

## 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). 2011



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### 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 (NaN<sub>3</sub>) 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)<sup>5</sup>
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 into contact with sensitive areas, wash with copious amounts of water.<sup>6</sup>
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. Campana D, *et al.* The cytoplasmic expression of CD3 antigens in normal and malignant cells of the T lymphoid lineage. *J Immunol.* 1987 Jan; 138(2):648-55.
2. Cabeçadas JM, Isaacson PG. Phenotyping of T-cell lymphomas in paraffin sections--which antibodies? *Histopathology.* 1991 Nov; 19(5):419-24.
3. Steward M, *et al.* Production and characterization of a new monoclonal antibody effective in recognizing the CD3 T-cell associated antigen in formalin-fixed embedded tissue. *Histopathology.* 1997 Jan; 30(1):16-22.
4. Fiehn AM, *et al.* CD3 immunohistochemical staining in diagnosis of lymphocytic colitis. *Hum Pathol.* 2016 Feb; 48(2):25-31.
5. 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."
6. 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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