

**CD3 T-Cell (M)**  
Concentrated and Prediluted Monoclonal Antibody  
Control Number: 901-110-032415

**ISO**  
**9001&13485**  
**CERTIFIED**

<b>Catalog Number:</b>	<b>CM 110 AK, BK, CK</b>	<b>PM 110 AA, H</b>	<b>IP 110 G10</b>	<b>OAI 110 T60</b>
<b>Description:</b>	0.1, 0.5, 1.0 ml, concentrated	6.0, 25 ml, prediluted	10 ml, prediluted	60 tests, prediluted
<b>Dilution:</b>	1:50 -1:100	Ready-to-use	Ready-to-use	Ready-to-use
<b>Diluent:</b>	Renoir Red	N/A	N/A	N/A

**Intended Use:**

For In Vitro Diagnostic Use  
CD3 T-Cell (M) [PS1] 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:**

Monoclonal antibody to human CD3, when used in conjunction with other antibodies, is regarded as a reliable pan T-cell antibody used in the immunophenotyping of lymphomas in paraffin sections. Most T-cell lymphomas show positivity for CD3. Notable exceptions include some of the more aggressive large T-cell lymphomas and anaplastic large cell (Ki-1, CD30) lymphomas, which may not express detectable antigen. CD3 immunoreactivity has also been reported in a minority of Reed-Sternberg cells of Hodgkin's disease and in some histiocytic tumors. CD3 expression of hemopoietic cells of the lymphoid, myeloid, and erythroid lineages in the human fetal and embryonic liver is rare. In a study of 50 archived T-cell lymphomas UCHL-1 (a monoclonal antibody to CD45RO) showed reactivity with 94% of cases, but lacked absolute specificity for T-cells, especially in high-grade lymphomas. CD3 showed reactivity with 80% of neoplastic cells, but with a higher specificity (1). When used in conjunction, UCHL-1 and monoclonal CD3 identified the majority of T-cell lymphomas in paraffin sections (2).

**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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** PS1

**Isotype:** IgG2a

**Total Protein Concentration:** ~10 mg/ml. Call for lot specific Ig concentration

**Epitope/Antigen:** CD3

**Cellular Localization:** Predominantly cell membrane. Some cytoplasmic

**Positive Tissue Control:** Tonsil or T-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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations (intelliPATH and manual use):**

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**Pretreatment Solution:** Borg or Reveal

**Pretreatment Protocol:**

Heat Retrieval Method: Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

**Protocol Recommendations (intelliPATH and manual use) Cont'd:**

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

**Primary Antibody:** Incubate for 30-45 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 Biocare's 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 has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection and intelliPATH Universal HRP Detection Kit. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. Use TBS for washing steps.

**intelliPATH™ Automated Slide Stainer:**

IP110 is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

**Protocol Recommendations (ONCORE Automated Slide Staining System):**

OAI110 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

**Protocol Name:** CD3

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

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR1, high pH; 101°C

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

**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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. 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.

**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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CERTIFIED**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) (3)
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. (4)
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/>.

**References:**

1. Cabecadas JM, Isaacson PG. Phenotyping of T-cell lymphomas in paraffin sections--which antibodies? *Histopathology*. 1991 Nov;19(5):419-24.
2. 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.
3. 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."
4. 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.

**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.