

CD3 T-Cell (M)

Concentrated and Prediluted Monoclonal Antibody
901-110-052523

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 110 AK, BK, CK	0.1, 0.5, 1.0 mL	1:100	Renoir Red
Predilute	PM 110 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IP 110 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 110 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 110 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 110 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	VP 110 G	6.0 mL	Ready-to-use	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 & 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. 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: PS1

Isotype: IgG2a

Protein Concentration: 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. 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):

VLTM110 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 (use at 1X).

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

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 Borg or Reveal Decloaker. Refer to the Borg or 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 30-45 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 Warp Red.

Counterstain:

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



60 Berry Drive
Pacheco, CA 94553
USA



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Protocol Recommendations (intelliPATH FLX and manual use)

Cont'd:

intelliPATH FLX Automated Slide Stainer:

IP110 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):

OAI110 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: 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

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

OPAI110 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

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Buffer Option): DS2-50

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

Block Option: Buffer

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

Protocol Recommendations (Ventana BenchMark XT / ULTRA):

VP110 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **ultraView on XT / ULTRA:**

Template/Detection: ultraView DAB

Pretreatment Protocol: ULTRA CC1 Standard

Primary Antibody: 32 minutes, 37°C

- Using **OptiView on ULTRA:**

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 32 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 16 minutes, 36°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.

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

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) (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>.

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. 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.

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60 Berry Drive
Pacheco, CA 94553
USA



Westervoortsedijk 60
6827 AT Arnhem
The Netherlands