

Pan Lymphoma Cocktail (LCA+CD20+CD3+CD43)

Prediluted Monoclonal Antibody Cocktail
901-3035-121917

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Catalog Number: API 3035 AA

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

Pan Lymphoma Cocktail (LCA+CD20+CD3+CD43) is intended for laboratory use in the qualitative identification of leukocyte cellular surface markers CD45, CD20, CD3, and CD43 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:

CD45 (LCA), CD20, CD3 and CD43 are specific leukocyte markers that have been routinely used in the identification and assessment of lymphoid neoplasms. The combination of antibodies to each of these cell surface molecules offers a broad-spectrum marker for the identification of a variety of leukocytes, which may be useful as a pan lymphoma marker.

CD45, leukocyte common antigen (LCA), belongs to the family of at least four isoforms of membrane glycoproteins (220, 205, 190, 180kDa) expressed on hematopoietic cell lines, but absent on non-hematopoietic cell lines and normal and malignant non-hematopoietic tissues. As a result, antibodies to CD45 have been shown to aid in the differential identification of lymphoid neoplastic cells from non-hematopoietic undifferentiated neoplasms. CD43 is a cell surface glycoprotein, which is expressed on all thymocytes and T-cells. CD43 is involved in activation of T-cells, B-cells, NK-cells, and monocytes. CD3 consists of five different polypeptide chains designated as gamma, delta, epsilon, zeta, and eta. The CD3 complex is closely associated at the lymphocyte cell surface with the T-cell antigen receptor. CD3 antigen is a highly specific marker for T-cells, and is present in majority of T-cell neoplasms, but is absent in B-cells. CD20 is a non-Ig differentiation antigen of B-cells and the expression of CD20 is restricted to normal and neoplastic B-cells, being absent from all other leukocytes and tissues.

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 secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Reagent Provided:

Pan Lymphoma Cocktail (LCA+CD20+CD3+CD43) is provided as a prediluted antibody cocktail of anti-CD45, anti-CD20, anti-CD3, and anti-CD43 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-CD45	anti-CD45	anti-CD20	anti-CD3	anti-CD43
Clone	PD7/26/16	2B11	L26	PS1	DF-T1
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal
Isotype	IgG1/kappa	IgG1/kappa	IgG2a/kappa	IgG2a	IgG1
Epitope/Antigen	CD45RB	CD45	CD20	CD3	CD43
Cellular localization	Cell surface	Cell surface	Cell surface	Cell surface	Cell surface

Storage and Stability:

Store at 2°C to 8°C. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human

Positive Tissue Control: Tonsil or B-cell and T-cell lymphomas

Protocol Recommendations:

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

Pretreatment: Perform heat retrieval using Biocare's Diva or Borg Decloaker. Refer to the Diva or Borg Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's 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 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 standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

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

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

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Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (15)

Precautions Cont'd:

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. (16)
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. Kurtin PJ, Pinkus GS. Leukocyte common antigen—a diagnostic discriminant between hematopoietic and nonhematopoietic neoplasms in paraffin sections using monoclonal antibodies: Correlation with immunologic studies and ultrastructural localization. *Hum Pathol.* 1985 Apr; 16(4):353-65.
2. Van Eyken P, *et al.* Expression of leukocyte common antigen in lymphoblastic lymphoma and small noncleaved undifferentiated non-Burkitt's lymphoma: an immunohistochemical study. *J Pathol.* 1987 Apr; 151(4):257-61.
3. Ozdemirli M, *et al.* Differentiating lymphoblastic lymphoma and Ewing's sarcoma: lymphocyte markers and gene rearrangement. *Mod Pathol.* 2001 Nov; 14(11):1175-82.
4. Biesemier KW, *et al.* A comparative study of frozen-section immunoperoxidase and flow cytometry for immunophenotypic analysis of lymph node biopsies. *Clin Diagn Lab Immunol.* 1994 May; 1(3):299-303.
5. Lucas DR, *et al.* Ewing sarcoma vs. lymphoblastic lymphoma: A comparative immunohistochemical study. *Am J Clin Pathol.* 2001 Jan; 115(1):11-7.
6. Olsen RJ, *et al.* Acute leukemia immunohistochemistry: a systematic diagnostic approach. *Arch Pathol Lab Med.* 2008 Mar; 132(3):462-75.
7. Carulli G, *et al.* Bone marrow infiltration in B-cell non-Hodgkin's lymphomas: comparison between flow cytometry and bone marrow biopsy. *Recent Prog Med.* 2005 June; 96(6):284-90.
8. Das DK. Serous effusions in malignant lymphomas: a review. *Diagn Cytopathol.* 2006 May; 34(5):335-47.
9. Saikia B, Gupta K, Saikia UN. The modern histopathologist: in the changing face of time. *Diagnostic Pathology.* 2008 Jun 6; 3:25.
10. 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.
11. Muzaffar S, *et al.* Immunophenotypic analysis of non-Hodgkin's lymphoma. *J Pak Med Assoc.* 1997 Apr; 47(4):106-9.
12. Nguyen DT, *et al.* Differential diagnosis of L26-positive, CD15-negative Hodgkin's disease and large B-cell lymphoma with a high content of reactive T-cells: a morphologic and immunohistochemical study. *Hematopathol Mol Hematol.* 1996; 10(3):135-50.
13. de Smet W, Walter H, van Hove L. A new CD43 monoclonal antibody induces homotypic aggregation of human leucocytes through a CD11a/CD18-dependent and -independent mechanism. *Immunology.* 1993 May; 79(1):46-54.

14. Basadonna GP, *et al.* Antibody-mediated targeting of CD45 isoforms: a novel immunotherapeutic strategy. *Proc Natl Acad Sci U S A.* 1998 Mar 31; 95(7):3821-6.

References Cont'd:

15. 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."
16. 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.