

# Leukocyte Common Antigen (LCA) Cocktail

Concentrated and Prediluted Cocktail Antibody  
901-016-021122

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

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 016 AK, BK, CK	0.1, 0.5, 1.0 mL	1:100	Van Gogh Yellow
Predilute	PM 016 AA	6.0 mL	Ready-to-use	N/A
intelliPATH FLX	IP 016 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 016 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 016 T60	60 tests	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALI 016 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

Leukocyte Common Antigen (LCA) Cocktail [PD7/26+2B11] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of leukocyte cellular surface marker protein CD45 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:

Studies have shown CD45 recognizes an antigen found on lymphoid cells. Most neoplastic B-cells and T-cells stain positively in leukemia and in non-Hodgkin's lymphomas; whereas most neoplastic myeloid and erythroid cells are negative (1-3). Studies have also shown it is unreactive with epithelium and connective tissues. The PD7/26 and 2B11 antibody was included in the 4th International Workshop and was designated as CD45. It belongs to an LCA family of glycoproteins with molecular weights of 180, 190, 205 and 220. It is well suited for formalin-fixed paraffin-embedded tissues and is used as a pan lymphocyte screener for lymphoma (1-3).

## 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- or two-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. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** PD7/26 and 2B11

**Isotype:** IgG1/kappa

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

**Epitope/Antigen:** CD45 (Leukocyte Common Antigen)

**Cellular Localization:** Cell surface

**Positive Tissue Control:** Tonsil or lymphoma

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative  
Van Gogh Yellow Diluent (PD902)

## 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 Reveal Decloaker. Refer to the 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 minutes at RT.

## Protocol Recommendations (intelliPATH FLX and manual use)

### Cont'd:

**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.

### intelliPATH FLX Automated Slide Stainer:

IP016 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 Notes:

1. This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.
2. A standard PBS diluent (pH 7.2-7.4) is not recommended for this antibody.

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

OAI016 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:** LCA

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

**Dewaxing (DS Option):** DS2

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

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

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

OPAI016 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:** LCA

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

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

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

**Reagent Name, Time, Temp.:** LCA, 20 min., 25°C

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

ALI016 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:** 10 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



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## 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](http://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 (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) (4)
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. (5)
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. Muzaffar S, *et al.* Immunophenotypic analysis of non-Hodgkin's lymphoma. JPMMA J Pak Med Assoc. 1997 Apr; 47(4):106-9.
2. Michels S, *et al.* Immunostaining for leukocyte common antigen using an amplified avidin-biotin-peroxidase complex method and paraffin sections. A study of 735 hematopoietic and nonhematopoietic human neoplasms. Arch Pathol Lab Med. 1987 Nov; 111(11):1035-9.
3. 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.
4. 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."
5. 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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