

Pan Cytokeratin Plus [AE1/AE3+8/18]

Concentrated and Prediluted Cocktail Antibody
901-162-052523

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Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 162 A, B, C	0.1, 0.5, 1.0 mL	1:100	Da Vinci Green
Predilute	PM 162 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IP 162 G10	10 mL	Ready-to-use	N/A
ONCORE	OAI 162 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 162 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 162 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 162 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For In Vitro Diagnostic Use

Pan Cytokeratin Plus [AE1/AE3+8/18] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of a broad spectrum of acidic and basic cytokeratin proteins 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:

AE1/AE3 recognizes acidic and basic subfamilies of cytokeratins. The cocktail of these two antibodies can be used to detect most human epithelia. The acidic cytokeratins have molecular weights of 56.5, 55, 51, 50, 48, 46, 45, and 40 kDa. The basic cytokeratins have molecular weights of 65-67, 64, 59, 58, 56 and 52 kDa. Clone 5D3 recognizes cytokeratin (CK) 8 and 18 intermediate filament proteins. These are 52.5 kDa and 45 kDa respectively. In normal tissues, 5D3 recognizes all simple and glandular epithelium. In the past, AE1/AE3 has had problems marking certain tissues types and adenocarcinomas. The addition of CK 8/18 remedies some of these problems. For example, a study of twenty-eight lipid cell (steroid cell) tumors of the ovary were studied by immunohistochemistry; 46% were positive for cytokeratin 8/18 antibody, 37% were positive with the cytokeratin cocktail AE1/AE3.

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, mouse and rat

Clone: AE1/AE3 + 5D3

Isotype: IgG1

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: AE1/AE3 + CK8/18

Cellular Localization: Cytoplasmic

Positive Tissue Control: Skin or adenocarcinoma

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 Solution (recommended): Reveal or Trypsin

Pretreatment Protocol:

Heat Retrieval Method:

Perform heat retrieval using Biocare's Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.

Digestion Method: Digest with Trypsin enzyme for 5 minutes at 37°C - or - for 20 minutes at RT.

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.

intelliPATH FLX Automated Slide Stainer:

IP162 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 pretreatment.

Technical Notes:

1. With cytokeratin markers, heat retrieval may provide a higher sensitivity assay; whereas, enzyme digestion may produce greater specificity. Users should validate the pretreatment method for their specific application.

2. 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):

OAI162 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: Pan CK Plus

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Option): DS Buffer

Antigen Retrieval (AR Option): AR2, low pH; 90°C

Reagent Name, Time, Temp.: Pan CK Plus, 30 min., 25°C

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

OPAI162 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: Pan CK Plus

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Buffer Option): DS Buffer

Antigen Retrieval (AR Option): AR2, low pH; 90°C

Block Option: Buffer

Reagent Name, Time, Temp.: Pan CK Plus, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI162 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 32 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 16 minutes, 36°C

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

ALI162 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

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) (6)

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

Precautions Cont'd:

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. (7)

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. Seidman JD, Abbondanzo SL, Bratthauer GL. Lipid cell (steroid cell) tumor of the ovary: immunophenotype with analysis of potential pitfall due to endogenous biotinlike activity. *Int J Gynecol Pathol.* 1995 Oct; 14(4):331-8.

2. Bunton TE. The immunocytochemistry of cytokeratin in fish tissues. *Vet Pathol.* 1993 Sep; 30(5):418-25.

3. Sorensen SC, *et al.* Structural distinctions among human breast epithelial cells revealed by the monoclonal antikeratin antibodies AE1 and AE3. *J Pathol.* 1987 Oct; 153(2):151-62.

4. Pinkus GS, Etheridge CL, O'Connor EM. Are keratin proteins a better tumor marker than epithelial membrane antigen? A comparative immunohistochemical study of various paraffin-embedded neoplasms using monoclonal and polyclonal antibodies. *Am J Clin Pathol.* 1986 Mar; 85(3):269-77.

5. Pinkus GS, *et al.* Optimal immunoreactivity of keratin proteins in formalin-fixed, paraffin-embedded tissue requires preliminary trypsinization. An immunoperoxidase study of various tumours using polyclonal and monoclonal antibodies. *J Histochem Cytochem.* 1985 May; 33(5):465-73.

6. 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."

7. 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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