

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

Concentrated and Prediluted Cocktail Antibody
902-162-060922

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 162 A, B, C	0.1, 0.5, 1.0 mL	1:100	Da Vinci Green
Predilute	APR 162 AA, H	6.0, 25 mL	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALR 162 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind 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.

Staining 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.

Staining 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.

Technical Note:

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.

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

ALR162 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:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 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.



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Precautions Cont'd:

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>.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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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