

Cytokeratin LMW (8/18)

Concentrated and Prediluted Monoclonal Antibody
902-056-081921

BIOCARE
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 056 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	APR 056 AA, H	6.0, 25 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 056 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALR 056 G7	7.0 mL	Ready-to-use	N/A

Intended Use:

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

Summary and Explanation:

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 (1,2). In neoplastic tissues, 5D3 may prove useful for the identification of adenocarcinomas and some squamous cell carcinomas (1). Use in conjunction with HMW CK to rule out squamous cell carcinoma. Keratinizing squamous carcinomas are generally negative. It works well in formalin-fixed, paraffin-embedded tissue 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 (does not stain mouse and rat)

Clone: 5D3

Isotype: IgG1

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: CK 8/18 (LMW)

Cellular Localization: Cytoplasmic

Positive Tissue Control: Skin

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 Protocol:

Pretreatment may be performed by heat retrieval or enzyme digestion.

Heat Retrieval Method:

Perform heat retrieval using Diva or Reveal Decloaker. Refer to the Diva or Reveal Decloaker product data sheet for specific instructions.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:

Digestion Method:

Digest with Pepsin enzyme for 5 minutes at 37°C – OR – for 15 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.

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.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):

AVR056 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

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

ALR056 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 ER2

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:

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) (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 in 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>.

Technical Support:

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

References:

1. Angus B, *et al.* Cytokeratins in cervical dysplasia and neoplasia: a comparative study of immunohistochemical staining using monoclonal antibodies NCL-5D3, CAM5.2, and PKK1. *J Pathol.* 1988 May;155(1):71-5.
2. Angus B, *et al.* NCL-5D3: a new monoclonal antibody recognizing low molecular weight cytokeratins effective for immunohistochemistry using fixed paraffin-embedded tissue. *J Pathol.* 1987 Dec;153(4):377-84.
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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