

E-Cadherin (RM)

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3012-032619

BIOCARE
M E D I C A L

Catalog Number:	ACI 3012 A, C	API 3012 AA	VLTR 3012 G20
Description:	0.1, 1.0 mL, conc.	6.0 mL, RTU	20 mL, RTU
Dilution:	1:50	Ready-to-use	Ready-to-use
Diluent:	Renaissance Background Reducing	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

E-Cadherin (RM) [EP6] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of E-cadherin protein 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:

E-Cadherin is a transmembrane glycoprotein that plays a key role in cell-cell adhesion in epithelial tissues (1-2). The adherens junction between epithelial cells is comprised of extracellular domains of E-Cadherin from adjacent cells, which interact through a molecular zipper motif. In normal tissues, immunostaining of E-Cadherin is localized to the membrane of epithelial cells, consistent with its role in cell adhesion.

Immunohistochemical studies have shown E-Cadherin to be expressed in breast ductal carcinoma with loss of expression in lobular carcinoma (1-2). As a result, mouse monoclonal anti-E-Cadherin [HECD-1] has been used by pathologists to differentiate between ductal and lobular carcinomas of the breast, with currently published sensitivity and specificity of approximately 90% (3). Rabbit monoclonal E-Cadherin antibody may combine the best properties of both monoclonal antibodies and rabbit antisera.

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: Rabbit monoclonal

Species Reactivity: Human; others not tested

Clone: EP6 (previously known as EP700Y)

Isotype: IgG

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: E-Cadherin

Cellular Localization: Membrane

Positive Tissue Control: Normal breast or breast ductal cell carcinoma

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 (VALENT® Automated Slide Staining Platform):

VLTR3012 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

Deparaffinization: Deparaffinize for 8 minutes with Val DePar.

Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.

Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.

Primary Antibody: Incubate for 20 minutes.

Secondary: N/A

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 20 minutes with Val Universal Polymer.

Chromogen: Incubate for 5 minutes with Val DAB.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated solution if using Decloaking Chamber Pro or Decloaking Chamber Plus. If using Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated 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:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 detection system. Use TBS for washing steps.

Performance Characteristics:

Rabbit monoclonal E-Cadherin was negative in all breast lobular carcinomas (n=29) and was 100% concordant in ductal cell carcinomas when compared to clone [HECD-1] (n=81); however, staining intensity was significantly increased in all ductal cell carcinomas with the rabbit monoclonal anti-E-Cadherin.

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

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Limitations Cont'd:

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) (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. de Deus Moura R, *et al.* Immunohistochemistry applied to the differential diagnosis between ductal and lobular carcinoma of the breast. *Appl Immunohistochem Mol Morphol.* 2013 Jan;21(1):1-12.
2. Dabbs DJ, Bhargava R, Chivukula M. Lobular versus ductal breast neoplasms: the diagnostic utility of p120 catenin. *Am J Surg Pathol.* 2007 Mar;31(3):427-37.
3. Moriya T, *et al.* The role of immunohistochemistry in the differential diagnosis of breast lesions. *Pathology.* 2009 Jan;41(1):68-76.
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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