

Epithelial Membrane Antigen (EMA) [E29]

Concentrated and Prediluted Monoclonal Antibody
901-3038-011118

BIOCARE
M E D I C A L

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|------------------------|---------------------------|--------------------|
| Catalog Number: | ACI 3038 A, C | API 3038 AA |
| Description: | 0.1, 1.0 ml, concentrated | 6.0, prediluted |
| Dilution: | 1:100 | Ready-to-use |
| Diluent: | Da Vinci Green | N/A |

Intended Use:

For In Vitro Diagnostic Use

Epithelial Membrane Antigen (EMA) [E29] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of epithelial membrane antigen 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:

Epithelial membrane antigen (EMA) belongs to a heterogeneous family of highly glycosylated transmembrane proteins known as human milk fat globule (HMFG) membrane proteins. This family of antigens is not restricted to breast but may also be found in secretory epithelial cells, to a lesser degree, in non-secretory epithelium (e.g., squamous epithelium) and rarely in non-epithelial cells. EMA is best considered a broad-spectrum antibody that is reactive against many types of adenocarcinoma. Breast and skin adnexal tumors are strongly positive. A lesser degree of staining is seen in carcinomas of the endometrium, kidney, thyroid, stomach, pancreas, lung, colon, ovary, prostate and cervix. Embryonal carcinomas, medullary carcinomas of thyroid, squamous carcinomas, sarcomas, lymphomas, and melanomas all tend to be nonreactive or show rare positive cells. Transitional cell carcinomas may show weak reactivity and anaplastic large cell lymphomas can be positive for EMA.

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 secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: E29

Isotype: IgG2a

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Epithelial membrane antigen

Cellular Localization: Cell membrane and cytoplasmic

Positive Tissue Control: Colon and breast cancer

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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

Protocol Recommendations Cont'd:

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

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's 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 Biocare's 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. Do not allow tissue sections to dry during the staining procedure. Dried tissue sections may display increased non-specific or uneven staining.

2. This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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) (5)

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. (6)

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Precautions Cont'd:

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. Verdu M, *et al.* Clinicopathological and molecular characterization of colorectal micropapillary carcinoma. *Mod Pathol.* 2011 May;24(5):729-38.
2. Saad RS, *et al.* The value of epithelial membrane antigen expression in separating benign mesothelial proliferation from malignant mesothelioma: a comparative study. *Diagn Cytopathol.* 2005 Mar;32(3):156-9.
3. Carbone A, Gloghini A, Volpe R. Immunohistochemistry of Hodgkin and non-Hodgkin lymphomas with emphasis on the diagnostic significance of the BNH9 antibody reactivity with anaplastic large cell (CD30 positive) lymphomas. *Cancer.* 1992 Dec 1;70(11):2691-8.
4. Heyderman E, *et al.* A new monoclonal antibody to epithelial membrane antigen (EMA)-E29. A comparison of its immunocytochemical reactivity with polyclonal anti-EMA antibodies and with another monoclonal antibody, HMFG-2. *Br J Cancer.* 1985 Sep;52(3):355-61.
5. 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."
6. 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.