Epithelial Membrane Antigen (EMA [Mc-5])
Concentrated and Prediluted Monoclonal Antibody
Control Number: 901-143-090517

<table>
<thead>
<tr>
<th>Catalog Number:</th>
<th>CM 143 A, B, C</th>
<th>PM 143 AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description:</td>
<td>0.1, 0.5, 1.0 ml, concentrated</td>
<td>6.0 ml, prediluted</td>
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<tr>
<td>Dilution:</td>
<td>1:150-1:250</td>
<td>Ready-to-use</td>
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<tr>
<td>Diluent:</td>
<td>Da Vinci Green</td>
<td>N/A</td>
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**Intended Use:**
For In Vitro Diagnostic Use

**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 nonsecretory epithelium (e.g., squamous epithelium) and rarely in nonepithelial cells. EMA is best considered a broad-spectrum antibody that is reactive against many types of adenocarcinoma. EMA can differentiate between the origins of glandular organs. 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. Note that the cells of anaplastic large cell lymphoma are positive for EMA in a minority of cases. EMA or Leu-M1 positivity, when coupled with CEA positivity, strongly favors metastasis to the liver.

**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:** Mc-5

**Isotype:** IgG,

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

**Epitope/Antigen:** EMA

**Cellular Localization:** Cytoplasmic and cell membrane

**Positive Control:** Breast carcinoma

**Normal Tissue:** Breast

**Abnormal Tissue:** Pancreas and lung adenocarcinomas, 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 Peroxidazed 1.

** Pretreatment Solution:** Reveal

** Pretreatment Protocol:**
Heat Retrieval Method: Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

** Protein Block:**
Optional: Incubate for 10-15 minutes at RT with Biocare's Background Sniper.

** Primary Antibody:** Incubate for 30-45 minutes at RT

** Probe:** Incubate for 10 minutes at RT with a Probe.

** Polymer:** Incubate for 10-20 minutes at RT with a Polymer.

** Protocol Recommendations Cont'd:**

** Chromogen:**
Incubate for 5 minutes at RT when using Biocare's DAB. - OR - Incubate for 10-20 minutes at RT when using Biocare's Vulcan Fast Red.

** Counterstain:**
Counterstain with Hematoxylin. Rinse with deionized water. Apply Tacha's Bluin solution for 1 minute. Rinse with deionized water.

** Technical Note:**
This antibody has been standardized with Biocare's MACH 4 detection system. It can also be used on an automated staining system and with other Biocare polymer detection kits. 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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

**Quality Control:**
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about tissue controls.

**Precautions:**
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.

** Provided:**
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)

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.

**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: