Mammaglobin, RMab
Clone: EP249
Rabbit Monoclonal

Inset: IHC of Mammaglobin on a FFPE Breast Tissue

Intended Use
For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The Mammaglobin antibody, clone EP249, has been manufactured using Epitomics RabMab® technology covered under Patent No. ’s 5,675,063 & 7,402,409.

Immunogen
A synthetic peptide corresponding to residues of human Mammaglobin A protein.

Summary and Explanation
Mammaglobin is a gene that encodes a 10 kDa glycoprotein. In humans, expression of the gene is limited to the adult mammary gland. A correlation between increased expression of the gene and Breast Cancer has been reported. Mammaglobin mRNA is present in high levels in human Breast Cancer cell lines and primary Breast Cancers. High levels of mRNA have been detected in normal human sweat glands as well, but are absent in Sweat Gland Tumors.

Anti-Mammaglobin (EP249) has been shown to be effective in detecting up to 85% of Breast Carcinomas using immunohistochemical techniques. Studies investigating the detection of mRNA by RT PCR from circulating carcinoma cells in the peripheral blood of Breast Cancer patients have shown that mammaglobin is a highly-specific marker and correlates with several prognostic factors, such as lymph node involvement.

Presentation
Anti-Mammaglobin (EP249) is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Antibody Type</th>
<th>Dilution</th>
<th>Volume/Qty</th>
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<tbody>
<tr>
<td>BSB 5743</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
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<td>BSB 5744</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
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<td>BSB 5745</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
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<tr>
<td>BSB 5746</td>
<td>Concentrated</td>
<td>1:25 - 1:100</td>
<td>0.1 mL</td>
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<tr>
<td>BSB 5747</td>
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<td>1:25 - 1:100</td>
<td>0.5 mL</td>
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<tr>
<td>BSB 5748</td>
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<td>1.0 mL</td>
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Control Slides Available

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>BSB 5749</td>
<td>5 slides</td>
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</table>

Storage
Store at 2-8°C (Control Slides: Store at 20-25°C)

Stability
This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Precautions
1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, “Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories” (see References in this document).
Staining Procedure
1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:
   a. TintoRetriever Pressure Cooker or Equivalent
      Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.
   b. TintoRetriever PT Module or Water Bath Method
      Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.
   c. Conventional Steamer Method
      Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.
6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer’s instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

<table>
<thead>
<tr>
<th>Step</th>
<th>ImmunoDetector AP/HRP</th>
<th>PolyDetector AP/HRP</th>
<th>PolyDetector Plus HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase/HP Blocker</td>
<td>5 min.</td>
<td>5 min.</td>
<td>5 min.</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
</tr>
<tr>
<td>Substrate-Chromogen</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
</tr>
<tr>
<td>Counterstain/Coverslip</td>
<td>Varies</td>
<td>Varies</td>
<td>Varies</td>
</tr>
</tbody>
</table>

Mounting Protocols
For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

Product Limitations
Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References