**p120 + E-cadherin**

Prediluted Multiplex Antibody Reagent

**Catalog Number:** API 3011 DS AA  
**Control Number:** 901-3011DS-090717

**Intended Use:**
For In Vitro Diagnostic Use.

p120 + E-cadherin is intended for laboratory use in the qualitative identification of p120 and E-cadherin proteins 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 patients clinical history and other diagnostic tests by a qualified pathologist.

**Summary and Explanation:**
Diagnostic reproducibility of lobular vs. ductal lesions, based on histology alone, is less than optimal. The proper distinction between atypical lobular hyperplasia, lobular carcinoma in situ and low-grade ductal carcinoma in situ is critical for patient management. Studies have shown E-cadherin, a negative membrane marker for lobular neoplasia, is useful in the distinction of ductal neoplasia vs. lobular; however as a negative marker for lobular carcinoma, it can be difficult to interpret, particularly in challenging cases (2). Studies have shown accurate categorization of ductal vs. lobular neoplasia in the breast with a cocktail of p120 + E-cadherin and helped give further clarification in the separation of low-grade ductal carcinoma in situ from lobular neoplasia. Of other tumors that may morphologically mimic lobular carcinoma, diffusely infiltrating variants of rectal and gastric carcinomas have shown diffuse cytoplasmic p120 immunostaining (1-2). This Multiplex IHC stain may enable the identification of the presence and the extent of the lobular lesions due to its bright pink color in lobular lesions, and therefore may aid in providing a more accurate diagnosis.

**Principle of Procedure:**
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

**Reagent Provided:**
p120 + E-cadherin is provided as a prediluted antibody cocktail of anti-p120 and anti-E-cadherin antibodies, in buffer with carrier protein and preservative.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>anti-p120</th>
<th>anti-E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone</td>
<td>96/p120</td>
<td>EP6*</td>
</tr>
<tr>
<td>Source</td>
<td>Mouse monoclonal</td>
<td>Rabbit monoclonal</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
<td>IgG</td>
</tr>
<tr>
<td>Epitope/Antigen</td>
<td>p120/catenin</td>
<td>E-cadherin</td>
</tr>
<tr>
<td>Cellular Localization</td>
<td>Cytoplasmic</td>
<td>Membrane</td>
</tr>
<tr>
<td>Staining</td>
<td>Red (Warp Red)</td>
<td>Brown (DAB)</td>
</tr>
</tbody>
</table>

*Previously known as EP700Y

**Storage and Stability:**
Store at 2°C to 8°C. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues).

**Species Reactivity:** Human, others not tested

**Positive Tissue Control:** Breast cancer

**Protocol Recommendations:**
- **Deparaffinization and rehydration:** Perform deparaffinization of tissues with xylene or xylene substitute, followed by rehydration through graded alcohols.
- **Peroxide Block:** Block for 5 minutes with Biocare's Peroxidazed 1.
- **Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
- **Protein Block:** Incubate for 10 minutes at RT with Biocare's Background Punisher.
- **Primary Antibody:** Incubate for 30 minutes at RT.
- **Double Stain Detection:** Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 1.
- **Chromogen (1):** Incubate for 5 minutes at RT with Biocare's Betazoid DAB.
- **Chromogen (2):** Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.
- **Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

**Technical Notes:**
This antibody has been standardized with Biocare's MACH 2 Double Stain 1 detection system. It can also be used on an automated staining 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:**

**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...
p120 + E-cadherin
Prediluted Multiplex Antibody Reagent
Control Number: 901-3011DS-090717

Precautions Cont’d:
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 in 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:

Produced using Abcam’s RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.