CK HMW + p63 + AMACR (RM)
Prediluted Multiplex Antibody Reagent
Control Number: 901-3154DS-090917

Catalog Number: API 3154DS AA, H
Description: 6.0, 25 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Catalog Number: IPI 3154DS G10
Description: 10 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use
CK HMW + p63 + AMACR (RM) is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of high molecular weight cytokeratin (CK 1, 5, 10, 14), p63 and AMACR 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 patient’s clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
High molecular weight cytokeratins are expressed in a variety of normal and neoplastic epithelial tissues (1). In prostate, CK HMW [34BE12] has been shown to be a useful marker of basal cells of normal glands and prostatic intraepithelial neoplasia (PIN), a precursor lesion to prostatic adenocarcinoma; whereas invasive prostatic adenocarcinoma typically lacks a basal cell layer (2-4).
p63, a homolog of the tumor suppressor p53, has been identified in proliferating basal cells in the epithelial layers of a variety of tissues, including epidermis, cervix, urothelium and prostate (5). p63 was detected in nuclei of the basal epithelium in normal prostate glands; however, it was not expressed in malignant tumors of the prostate (6).

α-Methylacyl coenzyme A racemase (AMACR), also known as P504S, is a peroxisomal and mitochondrial enzyme that plays a role in bile acid synthesis and β-oxidation of branched chain fatty acids (7). AMACR was initially identified from a cDNA library as a gene that is overexpressed in human prostate cancer; with little or no expression in normal prostate (8,9). In immunohistochemistry, AMACR has been shown to be a specific marker of prostatic adenocarcinoma (8-11). Additionally, prostate glands involved in PIN have been found to express AMACR, whereas AMACR was nearly undetectable in benign glands (11,12).

Studies have shown that combinations of CK HMW [34BE12], p63, and/or AMACR may be useful in the evaluation of normal prostate glands, PIN and prostatic adenocarcinoma (13,14). U.S. Patent 8,603,765 and patents pending.

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:
CK HMW + p63 + AMACR (RM)* is provided as a prediluted antibody cocktail of anti-CK HMW, anti-p63 and anti-AMACR antibodies in buffer with carrier protein and preservative. *Product formerly known as PIN-4®

<table>
<thead>
<tr>
<th>Antibody</th>
<th>anti-CK HMW</th>
<th>anti-p63</th>
<th>anti-AMACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone</td>
<td>34BE12</td>
<td>4A4</td>
<td>13H4</td>
</tr>
<tr>
<td>Source</td>
<td>Mouse</td>
<td>Mouse</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG1/kappa</td>
<td>IgG2a/kappa</td>
<td>IgG</td>
</tr>
<tr>
<td>Epitope/Antigen</td>
<td>CK HMW</td>
<td>p63</td>
<td>AMACR</td>
</tr>
<tr>
<td>Cellular Localization</td>
<td>Cytoplasmic</td>
<td>Nuclear</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>Staining</td>
<td>Brown (DAB)</td>
<td>Brown (DAB)</td>
<td>Red (Warp Red)</td>
</tr>
</tbody>
</table>

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: Normal prostate and prostatic adenocarcinoma

Protocol Recommendations (intelliPATH and manual use):

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


 Primary Antibody: 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 2.

 Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betadoid 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.

 intelliPATH Automated Slide Stainer:
IP3154DS is intended for use on the intelliPATH™ Automated Slide Stainer. Refer to the intelliPATH Automated Slide Stainer manual for specific instructions on its use. When using the intelliPATH, peroxide block with intelliPATH Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

Protocol Recommendations (ONCORE Automated Slide Staining System):
OA13154 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

Protocol Name: HMW p63 AMACR

Protocol Template (Description): Multiplex 2 Template 1

Dewaxing (DS Option): DS Buffer

Antigen Retrieval (AR Option): AR2, low pH; 101°C

Reagent Name, Time, Temp.: HMW p63 AMACR, 30 min., 25°C
**Technical Notes:**

This antibody has been optimized for use with Biocare's MACH 2 Double Stain, intelIPATH Multiplex Secondary Reagent 2 and ONCORE Multiplex Detection 2. 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 (Na₃N) 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) (15)

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. (16)

3. Microbial contamination of reagents may result in an increase in nonspecific staining. 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:**


