CK HMW + p63 + AMACR (RM)

Prediluted Multiplex Antibody Reagent
901-3154DS-010323

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Q Series– For Leica BOND-III	ALI 3154DS G7	7.0 mL	Ready-to-use	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 [34βE12] 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 [34βE12], 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 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®

Antibody	anti-CK HMW	anti-p63	anti-AMACR
Clone	34βE12	4A4	13H4
Source	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG2a/kappa	IgG
Epitope/ Antigen	CK HMW	p63	AMACR
Cellular Localization	Cytoplasmic	Nuclear	Cytoplasmic
Staining	Brown (DAB)	Brown (DAB)	Red

Storage and Stability:
Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Normal prostate and prostatic adenocarcinoma

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3154DS is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Protocol Name: Parallel DS Protocol K
Detection: ChromoPlex 1 Dual IHC
HIER: 20 min with ER2
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Polymer mHRP: 8 min
Polymer rAP: 20 min
Mixed DAB Refine: 5 min
Mixed Red Refine 2: 10 + 5 min
Hematoxylin: 5 min

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.

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 (NaN_3) 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]

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Precautions Cont'd:
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.[16]
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:
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8. Xu J, *et al.* Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. Cancer Res. 2000 Mar 15; 60(6):1677-82.
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15. 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."
16. 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.

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