ERG + AMACR

Prediluted Multiplex IHC Cocktail

Control Number: 902-3013DS-090414

Catalog Number: **APR 3013 DS AA**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

Summary and Explanation:

In human prostate cancer, the ERG oncogene is frequently overexpressed due to chromosomal translocations involving ERG and regulatory sequences of the TMPRSS2 or other androgen responsive genes. The mouse monoclonal anti-ERG antibody shows an unprecedented 99.9% specificity for detecting prostatic adenocarcinoma. The report shows strong correlation between the expression of the ERG protein and the presence of TMPRSS2:ERG rearrangement and a remarkable concordance (96.5%) of ERG positive prostatic intraepithelial neoplasia (PIN) and ERG positive carcinoma in prostatectomy specimens.

Therefore, as a hallmark of the TMPRSS2:ERG chromosomal translocation, ERG expression offers a rare, but definitive marker of adenocarcinoma of prostatic origin, and unique opportunities to indicate oncogenic activations in PIN, to stratify prostate cancer patients for ERG oncogene status and to monitor treatment efficacy.

AMACR (P504S) protein is expressed in prostatic adenocarcinoma, but not in benign prostatic tissue. It has also been found to be expressed in some premalignant lesions of the prostate: high-grade prostatic intraepithelial neoplasia (PIN) and atypical adenomatous hyperplasia. Studies have also documented AMACR can be used as a positive marker for prostate cancer and may be useful to confirm small foci of prostate carcinoma in needle biopsies. AMACR stains the majority of prostate cancer; however, AMACR has been shown to stain many other types of carcinomas such as hepatoma, breast carcinoma, pancreatic and islet tumors.

Note: *ERG [9FY] was developed by the Center for Prostate Disease Research in association with the Henry M. Jackson Foundation, Rockville, Maryland. U.S. Patent 8,765,916 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.

Source: Mouse monoclonal and rabbit monoclonal
Species Reactivity: Human; others not tested
Clone: 9FY + 13H4
Isotype: IgG1 + IgG
Epitope/Antigen: ERG and AMACR
Cellular Localization:
ERG (Nuclear): Brown with MACH 2 Double Stain 2 or red with MACH 2 Double Stain 1.
AMACR (Cytoplasmic): Red with MACH 2 Double Stain 2 or brown with MACH 2 Double Stain 1.
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration
Positive Control: ERG positive prostate cancer with normal and/or PIN glands
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues).
Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2ºC to 8ºC. 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.

Staining Protocol Recommendations:

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

Pretreatment Solution (recommended): Diva

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 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 or 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 Note:

1. ERG [9FY] is highly specific and does not stain lymphocytes.
2. ERG [9FY] has been shown to stain endothelial cells, which may serve as a convenient internal positive control in most tissue sections.
3. This antibody cocktail has been standardized with Biocare's MACH 2 Double Stain 2 and MACH 2 Double Stain 1. It can also be used on an automated staining system. Use TBS buffer for washing steps.

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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) is 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) (9)
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. (10)
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/.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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References:

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2. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008 Jul; 8(7):497-511.
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4. Mohamed AA, *et al.* Ets family protein, erg expression in developing and adult mouse tissues by a highly specific monoclonal antibody. J Cancer. 2010 Oct 25;1:197 -208.
5. Miettinen M, *et al.* ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. Am J Surg Pathol. 2011 Mar; 35 (3):432-41.
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7. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. Semin Diagn Pathol. 2005 Feb; 22(1):88-104.
8. Trpkov K, Bartczak-McKay J, Yilmaz A. Usefulness of cytokeratin 5/6 and AMACR applied as double sequential immunostains for diagnostic assessment of problematic prostate specimens. Am J Clin Pathol. 2009 Aug; 132(2):211-20.
9. 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."
10. 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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