ERG (M), 2X

Prediluted Monoclonal Antibody

Control Number: 901-3017-092017

Catalog Number: **API 3017 AAK**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use

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. In particular, the TMPRSS2:ERG fusion gene has recently been found to be the most frequent gene rearrangement in prostate cancers, occurring in 45-65% of North American patients.

Recently, a mouse monoclonal anti-ERG antibody was developed with 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. Given the ease of performing IHC vs. FISH, ERG protein expression in formalin-fixed paraffin-embedded (FFPE) tissues may be an extremely useful tool for the routine identification of the ERG gene rearrangement and diagnosis of prostatic adenocarcinoma.

Further utility for the mouse monoclonal anti-ERG antibody has been demonstrated recently in detecting endothelial malignancies, such as Kaposi sarcoma.

ERG (M), 2X may be combined with AMACR (RM), 2X to form a primary antibody cocktail (see technical notes).

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

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 9FY

Isotype: IgG1

Epitope/Antigen: ERG

Cellular Localization: Nuclear

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration

Positive Control: ERG positive prostate cancer and/or PIN glands.

Normal Tissue: Normal prostate (endothelial cells only)

Abnormal Tissue: ERG positive prostate cancer and/or PIN glands.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues).

Supplied As:

ERG (M), 2X (API3017AA) 6ml

Dropper Bottle (DB3017)

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.

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. (See Technical Note #5)

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a polymer.

Chromogen: Incubate for 5 minutes at RT when using Biocare's DAB – OR – Incubate for 5-7 minutes at RT when using Biocare's Warp Red.

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 has been standardized with Biocare's MACH 2 detection system. It can also be used on an automated staining system and with other Biocare polymer detection kits. Use TBS buffer for washing steps.
4. The combination of ERG (M) and AMACR (RM) may be extremely useful for diagnosing adenocarcinoma of prostatic origins. AMACR stains cytoplasm in prostate adenomatous hyperplasia; ERG stains the nuclei of epithelial cells having undergone chromosomal translocations. The fusion gene, TMPRSS2:ERG has been found to be the most frequent gene rearrangement in prostate cancers. In this instance, the ERG + AMACR Cocktail becomes a double stain procedure enabling unique opportunities to indicate oncogenic activations in prostatic intraepithelial neoplasia (PIN).
5. Biocare's ERG (M), 2X may be combined at a 1:1 (equal parts) with AMACR (RM), 2X (cat# APA3016) to obtain a primary antibody cocktail. AMACR (RM), 2X is a rabbit monoclonal (RM) antibody; therefore, Biocare's MACH 2 Double Stain 1 (cat# MRCT523) or 2 (cat# MRCT525) may be used for detection.

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

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:

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

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Precautions Cont'd:

Health, 1976)

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. 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. The MSDS is available upon request and is located at http://biocare.net/support/msds/.

References:

1. Petrovics G, Liu A, Shaheduzzaman S, Furasato B, Sun C, Chen Y, Nau M, Ravindranath L, Chen Y-D, Dobi A, Srikantan V, Sesterhenn IA, McLeod DG, Vahey M, Moul WJ, Srivastava S. Frequent over-expression of *ETS* related gene-1 *(ERG1)* in prostate cancer transcriptome. *Oncogene* 24, 3847-3852 (2005).

2. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. *Nat Rev Cancer* 8, 497-511 (2008).

3. Furusato B, Tan SH, Young D, Dobi A, Sun C, Mohamed AA, Thangapazham R, Chen Y, McMaster G, Sreenath T, Petrovics G, McLeod DG, Srivastava S, Sesterhenn IA. ERG oncoprotein expression in prostate cancer: clonal progression of ERG positive tumor cells and potential for ERG based stratification. *Prostate Cancer and Prostatic Diseases* 13, 228-237 (2010).

4. Mohamed AA, Tan S-H, Mikhalkevich N, Ponniah S, Vasioukhin V, Bieberich CJ, Sesterhenn IA, Dobi A, Srivastava S, Sreenath LT. Ets Family Protein, Erg Expression in Developing and Adult Mouse Tissues by a Highly Specific Monoclonal Antibody. *Journal of Cancer* 1, 197-208 (2010).

5. Miettinen M, Wang Z-F, Paetau A, Tan S-H, Dobi A, Srivastava S, Sesterhenn IA.: ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. *American Journal of Surgical Pathology* 35, 432-441 (2011).

6. Mohamed AA, Tan S-H, Sun C, Shaheduzzaman S, Hu Y, Petrovics G, Chen Y, Sesterhenn IA, Li H, Sreenath T, McLeod DG, Dobi A, Srivastava S. *ERG* oncogene modulates prostaglandin signaling in prostate cancer cells, *Cancer Biology and Therapy* 11, 410-417 (2011).

7. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. *Semin Diagn Pathol* 22, 88-104 (2005).

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* 132, 211-220 (2009).

9. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove

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.

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