ERG-2 (ERG + CK5)

Prediluted Multiplex Antibody Reagent
901-437DS-060223

BIOCARE MEDICAL

Catalog Number:	**API 437DS AA**	**VLTMR 437 G20**
Description:	6.0 mL, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
ERG-2 (ERG + CK5) is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of ERG and cytokeratin 5 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:
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 (1,3-6). The mouse monoclonal anti-ERG antibody shows an unprecedented 99.9% specificity for detecting prostatic adenocarcinoma (3). 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 (3).
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 (6).
CK5 is a type II intermediate filament protein. CK5 is expressed in basal layers of most epithelia including normal prostate and normal breast tissues. CK5 stains normal basal cell layers in prostate, benign prostate hyperplasia (BPH) and prostatic intraepithelial neoplasia (PIN) (7,8).
The combination of ERG and CK5 provides a unique stain that identifies the TMPRSS2:ERG chromosomal translocation in prostate cancer (brown); but also highlights PIN (red): thus helping to visualize ERG positive PINs.

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 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:
ERG-2 (ERG + CK5) is provided as a prediluted antibody cocktail of anti-ERG and anti-CK5 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-ERG	anti-CK5
Clone	9FY	EP42
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/Antigen	ERG	C-terminus of human CK5
Cellular Localization	Nuclear	Cytoplasmic
Staining	Brown (DAB)	Red

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: ERG positive prostate cancer with normal and/or PIN glands

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTMR437 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block: Incubate for 10 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Double Stain Detection: Incubate for 30 minutes using Val Plex 2.
Chromogen (1): Incubate for 5 minutes with Val DAB.
Chromogen (2): Incubate for 15 minutes with Val Fast Red.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block: Incubate for 10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Double Stain Detection: Incubate for 30 minutes at RT using MACH 2 Double Stain 2.
Chromogen (1): Incubate for 5 minutes at RT with Betazoid DAB.
Chromogen (2): Incubate for 5-7 minutes at RT with 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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
The Netherlands

Rev: 062117

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Technical Notes:

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, for intelliPATH FLX and manual use, has been standardized with MACH 2 Double Stain 2. Use TBS buffer for washing steps.
4. If the end-user desires to reverse colors, MACH 2 Double Stain 1 can be substituted; however, revalidation may be required by the end-user.

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) (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 into 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.

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:

1. Petrovics G, *et al.* Frequent overexpression of *ETS* related gene-1 *(ERG1)* in prostate cancer transcriptome. Oncogene. 2005 May 26;24(23):3847-52.
2. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008 Jul; 8(7):497-511.
3. Furusato B, *et al.* ERG oncoprotein expression in prostate cancer: clonal progression of ERG positive tumor cells and potential for ERG based stratification. Prostate Cancer Prostatic Dis. 2010 Sep; 13(3):228-37.
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.

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6. Mohamed AA, *et al. ERG* oncogene modulates prostaglandin signaling in prostate cancer cells. Cancer Biol Ther. 2011 Feb 15; 11(4):410-7.
7. 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.
8. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. Semin Diagn Pathol. 2005 Feb; 22(1):88-104.
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.

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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
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The Netherlands

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