BIOCARE MEDICAL

ISO 9001&13485 CERTIFIED

ERG-2 (ERG and CK5)

Prediluted Multiplex IHC

Control Number: 901-437DSV-090314

VP Echelon™ Series

Catalog Number: **AVI 437DSK G**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

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. 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.
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).
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. Patent Pending.*

Principle of Procedure:

A sequential double stain is used for the simultaneous detection of two different antigens within one tissue section. A primary antibody is applied to the tissue, followed by a horseradish peroxidase (HRP) detection system. A denaturing step is required to eliminate cross-reactivity from the application of the second detection system. A second primary antibody is then applied, followed by an alkaline phosphatase (AP) detection system. Visualization of antigens is achieved with DAB and Red chromogens.

Source: Mouse monoclonal and Rabbit monoclonal

Species Reactivity: Human, others not tested

Clone: 9FY and EP1601Y

Isotype: IgG1 and N/A

Epitope/Antigen: ERG and Cytokeratin 5

Cellular Localization:
ERG (Nuclear): Brown
CK5 (Cytoplasmic): Red

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

Normal Tissue: Normal prostate (CK5) with blood vessels (ERG stains endothelial cells of blood vessels)

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

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:
ERG (VP421G) 1 x 6ml
CK5 (AVI430G) 1 x 6ml
V-Blocker (BRI4001G) 2 x 6ml

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:

Using ***ultra*View™ Detection Kit**

NOTE: V-Blocker must be filled in a registered *Ancillary Inline* User *Fillable Dispenser* prior use. V-Blocker must be registered as an "Option" in order to properly use it.

Pretreatment Solution (recommended): CC1

Pretreatment Protocol: Standard
For immunohistochemistry, V-Blocker is recommended to be applied prior to any detection system.

Primary Antibody ERG (Cat# VP421): Incubate for 32 minutes with No Heat.

ultraBlock (BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user).

Denaturation: Incubate for 4 minutes at 90°C.

Double Stain Primary Antibody CK5 (Cat# AVI430): Incubate for 32 minutes at 37°C.

DS ultraBlock (BRI4001): Incubate for 4 minutes (with appropriate Option # registered as used before).

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. Biocare's VP Echelon Series of predilutes have been developed for use with Ventana® Medical Systems, BenchMark® XT Immunohistochemistry Staining System in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers.
4. Application of V-Blocker prior to any detection system is highly recommended for background reduction especially because granular precipitate may be observed with AP Red Detection.

Performance Characteristics:
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 listed are not applicable to other detection systems, as results may vary. 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) 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)
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/.

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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 overexpression 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. 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).
8. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. *Semin Diagn Pathol* 22, 88-104 (2005).
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-Third Edition CLSI document M29-A3 Wayne, PA (2005).

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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.

Limitations and Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

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