

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 421 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	APR 421 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 421 G	6.0 mL	Ready-to-use	N/A
Q Series- For Leica BOND-III	ALR 421 G7	7.0 mL	Ready-to-use	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. 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. Further utility of the mouse monoclonal anti-ERG antibody, 9FY, has been shown in detecting endothelial malignancies, including Kaposi sarcoma.

Note: Clone 9FY [U.S. Patent 8,765,916 and patents pending] was developed by the Center for Prostate Disease Research with the Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, Maryland, USA.

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 one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 9FY

Isotype: IaG1

Total Protein Concentration: Call for lot specific Ig concentration. Epitope/Antigen: N-terminal ERG (see Technical Notes)

Cellular Localization: Nuclear

Positive Tissue Control: ERG positive prostate cancer 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 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Staining Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidazed 1.

Pretreatment: Perform heat retrieval using Reveal or Diva Decloaker. Refer to the Reveal or Diva Decloaker product data sheet for specific instructions. Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30-60 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe. **Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

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Staining Protocol Recommendations (intelliPATH FLX® and manual use) Cont'd:

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB - OR -Incubate for 5-7 minutes at RT with 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, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS buffer for washing steps.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):

AVR421 is intended for use with the BenchMark ULTRA Slide Staining System. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: ultraView DAB

Pretreatment Protocol: ULTRA CC1 Standard

Primary Antibody: 32 minutes, No Heat

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

V-Blocker is recommended to be applied prior to any detection system.

- Protocol Recommendation for Ventana BenchMark XT available upon request.

Staining Protocol Recommendations (O Series - For Leica BOND-<u>III):</u>

ALR421 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: IHC Protocol F Detection: Bond Polymer Refine HIER: 30 min with ER1 Peroxide Block: 5 min Marker (Primary Antibody): 15 min Post Primary: 8 min Polymer: 8 min Mixed DAB Refine: 10 min Hematoxylin: 5 min

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 (NaN₃) 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) (8)

ERG

Concentrated and Prediluted Monoclonal Antibody 902-421-111621



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 in contact with sensitive areas, wash with copious amounts of water. (9)

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.

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. Rosen P, *et al.* Clinical potential of the ERG oncoprotein in prostate cancer. Nat Rev Urol. 2012 Feb 14;9(3):131-7.

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.

 Braun M, *et al.* ERG protein expression and genomic rearrangement status in primary and metastatic prostate cancer - a comparative study of two monoclonal antibodies. Prostate Cancer Prostatic Dis. 2012 Jun;15(2):165-9.
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.

6. 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.

7. Mohamed AA, *et al. ERG* oncogene modulates prostaglandin signaling in prostate cancer cells. Cancer Biol Ther. 2011 Feb 15;11(4):410-7.

8. 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."

9. 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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