

ERG

Concentrated and Prediluted Monoclonal Antibody
902-421-102717

BIOCARE
M E D I C A L

Catalog Number:	ACR 421 A, C	APR 421 AA	AVR 421 G
Description:	0.1, 1.0 ml, concentrated	6.0, prediluted	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use	Ready-to-use
Diluent:	Renoir Red	N/A	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.

The mouse monoclonal anti-ERG antibody, clone 9FY, shows an unprecedented 99.9% specificity for detecting prostatic adenocarcinoma. Independent reports demonstrate 97-100% 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, detection of ERG expression by 9FY 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. Towards the stratification of patients, comparative evaluations of ERG protein expression status with 9FY and TMPRSS2-ERG gene fusions in hormone-naïve and castration resistant prostate cancers have shown promise for defining a subgroup of cases with dispersed androgen signaling pathway.

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 of the mouse monoclonal anti-ERG antibody, 9FY, has been shown in detecting endothelial malignancies, including Kaposi sarcoma. Reports have also demonstrated the superior performance of 9FY in chromatin immunoprecipitation (ChIP), immunofluorescence (IF) and immunoblot assays.

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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 9FY

Isotype: IgG1

Total Protein Concentration: ~10 mg/ml. 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. 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Staining Protocol Recommendations (intelliPATH and manual use):

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

Pretreatment: Perform heat retrieval using Biocare's 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 Biocare's 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.

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

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Staining Protocol Recommendations (Ventana BenchMark ULTRA Slide Staining System):

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

Template: U ultraView DAB

Pretreatment Protocol: ULTRA CC1 Standard (64 min) at 95°C

Primary Antibody: Incubate for 32 minutes with 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.

Detection: *ultraView*

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. ERG [9FY] was raised against an N-terminal epitope. Thus, it recognizes most forms of ERG proteins, such as ERG8 encoded by the dominant ERG mRNAs in prostate cancer. In contrast, other antibodies raised exclusively against C-terminal epitopes of ERG do not recognize ERG8 and other cancer associated forms of ERG that lack C-terminal sequences.
4. This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.
5. Protocol Recommendation for Ventana BenchMark XT available upon request.

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.



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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)
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.
4. 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.
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.
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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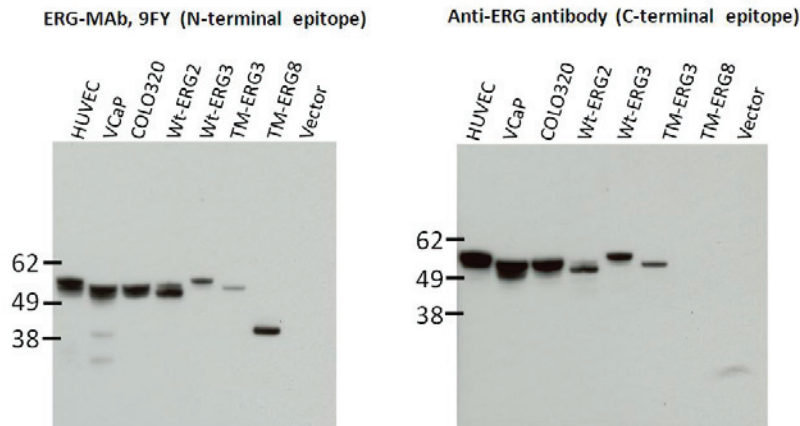
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Applications of anti-ERG [9FY]

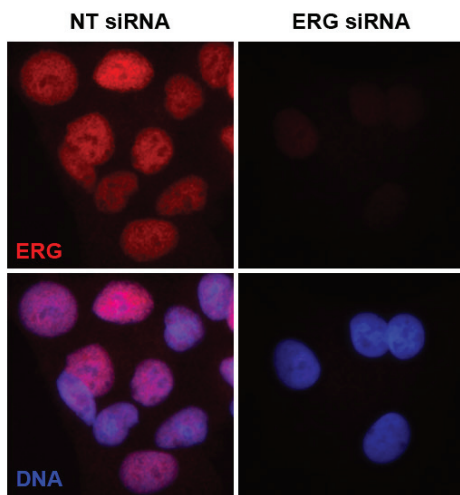
Western Blot, Immunofluorescence (IF) & Chromatin Immunoprecipitation (ChIP)

Western blot of 9FY



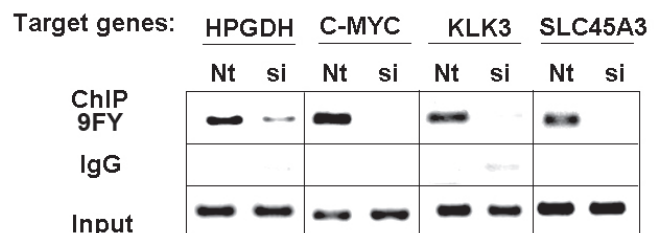
ERG-MAb, 9FY was raised against an N-terminal epitope. Thus, it recognizes most forms of ERG proteins, such as ERG8 encoded by the dominant ERG mRNAs in prostate cancer. In contrast, other antibodies raised exclusively against C-terminal epitopes of ERG do not recognize ERG8 and other cancer associated forms of ERG that lack C-terminal sequences.

Immunofluorescence using 9FY



Nuclear localization of endogenous ERG oncoprotein is shown by anti-ERG [9FY] in VCaP prostate cancer cells (ERG). Inhibition of ERG expression in response to ERG knockdown (ERG siRNA) demonstrates the specificity of anti-ERG [9FY]. In corresponding images below, nuclear staining of VCaP cells are visualized by DAPI.

Chromatin Immunoprecipitation using 9FY



Recruitment of endogenous ERG oncoprotein to regulatory regions of HPGD, c-MYC, KLK3(PSA) and SLC45A3(protein) genes in VCaP cells are shown by *in vivo* chromatin immunoprecipitation (ChIP) assay using anti-ERG [9FY]. Specificity of ERG recruitment is demonstrated by the knockdown of ERG binding by ERG siRNA. ChIP with IgG is shown as the negative control. Input indicates positive control genomic DNA amplicons.