ERG
Concentrated and Prediluted Monoclonal Antibody
901-421-102717

Catalog Number: CM 421 A, C PM 421 AA OAI 421 T60 VP 421 G
Description: 0.1, 1.0 ml, concentrated 6.0 ml, prediluted 60 tests, prediluted 6.0 ml, prediluted
Diluent: 1:100 Ready-to-use Ready-to-use N/A

Intended Use:
For In Vitro Diagnostic Use
ERG [9FY] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of ERG protein 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.

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-naive 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 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

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.

Protocol Recommendations (ONCORE Automated Slide Staining System):
Peroxide 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.

Protocol Recommendations (Ventana BenchMark ULTRA Slide Staining System):
ERG antibody, 9FY, has been shown to stain endothelial cells, which may serve as a convenient internal positive control in most tissue sections.

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.

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Technical Note Cont’d:
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 optimized for use with Biocare’s MACH 4 Universal HRP-Polymer Detection and ONCORE HRP Detection. Use TBS for washing steps.
5. Protocol Recommendation for Ventana BenchMark XT available upon request.

Quality Control:

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 (Na₃N₃) 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 into 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.

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixed, 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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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 Cont’d:

VP Echelon Series antibodies are developed solely by Biocare Medical LLC and do not imply approval or endorsement of Biocare’s antibodies by Ventana Medical Systems, Inc. Biocare and Ventana are not affiliated, associated or related in any way. Ventana®, BenchMark®, ultraView and OptiView are trademarks of Roche.
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(prostein) 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.

Images courtesy Dr. Shiv Srivastava, Center for Prostate Disease Research.

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