anti-ERG (EPR3864) Rabbit Monoclonal Primary Antibody

REF 790-4576

06478450001

IVD Σ 50

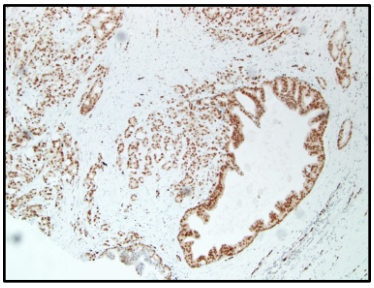

Figure 1. Prostate carcinoma positively staining with anti-ERG (EPR3864) antibody.

INTENDED USE

Anti-ERG (EPR3864) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of both wildtype ERG, and truncated ERG resulting from ERG gene rearrangement, by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

The anti-ERG (EPR3864) Rabbit Monoclonal Primary Antibody (anti-ERG (EPR3864) antibody) is a monoclonal antibody produced against the ERG protein encoded by the *ETS-related gene, ERG*. ERG is a 54kDa member of the E-26 transformation-specific (ETS) family of transcription factors.[1,2] Members of this family are nuclear DNA-binding phosphoproteins that regulate a variety of cellular processes including proliferation, differentiation, and apoptosis via activation or repression of several target genes. ERG is normally expressed in vascular endothelial cells and lymphocytes. In prostate carcinoma (PCa), the promoter region of *TMPRSS2,* an androgen-regulated and prostate-specific gene, is fused with the C-terminus of ERG, which results in overexpression of a truncated ERG protein.[3,4] Overexpression of truncated ERG is found in 40-70% of PCa, while it is generally absent in benign prostate.[4,5]

Several studies have demonstrated the presence of truncated ERG in approximately 50% PCa specimens.[5-8] Many studies have also shown high concordance between FISH-determined *ERG* gene rearrangement status and protein overexpression detected by ERG IHC.[5,9-14] Thus, IHC detection of ERG with anti-ERG (EPR3864) antibody may be used as an aid in the identification of prostate adenocarcinomas through the detection of truncated ERG.

The staining pattern for this antibody is nuclear. It may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

The anti-ERG (EPR3864) antibody binds to the C-terminus of the ETS transcription regulator ERG in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

Anti-ERG (EPR3864) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-ERG (EPR3864) antibody contains approximately 115 µg of a rabbit monoclonal antibody.

The antibody is diluted in a TBS buffer containing carrier protein.

Specific antibody concentration is approximately 23 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-ERG (EPR3864) antibody is a recombinant rabbit monoclonal primary antibody produced as a purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
16. General purpose laboratory equipment
17. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[15] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[16,17]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.

8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4576.

Table 1. Recommended staining protocol for anti-ERG (EPR3864) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild	ULTRA Cell Conditioning 1 Mild
Antibody (Primary)	16 minutes, 37 °C	32 minutes, 36 °C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[18]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-ERG (EPR3864) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of positive control tissue for this antibody is spleen (vessel endothelium).

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-ERG (EPR3864) antibody is predominantly strong nuclear staining with minimal cytoplasmic staining.

SPECIFIC LIMITATIONS

This antibody has an uncharacterized reactivity in lymphocytes, and is sensitive to fixation. Positive staining of vessel endothelium serves as an internal positive control of tissue reactivity. Anti-ERG (EPR3864) antibody demonstrates a known cross-reactivity with the FLI-1 protein, which does not interfere with the analysis of prostate samples.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for specificity, sensitivity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 2. Sensitivity/Specificity of anti-ERG (EPR3864) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	0/3
Adrenal gland	0/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Parathyroid gland	0/2	Liver	0/3
Pituitary gland	0/3	Salivary gland	0/3
Testis	0/3	Kidney	0/3
Thyroid	0/3	Prostate [a]	0/31
Breast	0/3	Endometrium	0/3
Spleen	3/3	Cervix	0/3
Tonsil	1/3	Skeletal muscle	0/2
Thymus	0/3	Skin	0/3
Bone marrow	0/3	Nerve	0/3
Lung	0/3	Mesothelium	0/3

[a] Tissues evaluated include normal and hyperplastic prostate

Table 3. Sensitivity/Specificity of anti-ERG (EPR3864) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1

Pathology	# positive / total cases
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Lobular carcinoma in situ (Breast)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinomas (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumor (GIST) (Colon)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	15/85
Urothelial carcinoma (Prostatic urethra)	0/1
Leiomyoma (Endometrium)	0/1
Adenocarcinoma (Endometrium)	0/1
Clear cell carcinoma (Endometrium)	0/1
Squamous cell carcinoma (Cervix)	0/2
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Mediastinum)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	0/1

Pathology	# positive / total cases
Hodgkin lymphoma (Lymph node)	0/1
Lymphoma, NOS (Lymph node)	2/2
B-cell Lymphoma, NOS	0/2
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Embryonal rhabdomyosarcoma (Soft tissue)	0/1
Leiomyosarcoma (Soft tissue)	0/1

Precision

Precision studies for anti-ERG (EPR3864) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of anti-ERG (EPR3864) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

1. Adamo P, Ladomery MR. The Oncogene ERG: A Key Factor in Prostate Cancer. Oncogene. 2016;35(4):403-414.
2. Gasi Tandefelt D, Boormans J, Hermans K, et al. ETS Fusion Genes in Prostate Cancer. Endocr Relat Cancer. 2014;21(3):R143-152..
3. Hermans KG, Boormans JL, Gasi D, et al. Overexpression of Prostate-Specific TMPRSS2(Exon 0)-ERG Fusion Transcripts Corresponds with Favorable Prognosis of Prostate Cancer. Clin Cancer Res. 2009;15(20):6398-6403.
4. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer. Science. 2005;310(5748):644-648.
5. van Leenders GJ, Boormans JL, Vissers CJ, et al. Antibody EPR3864 is Specific for ERG Genomic Fusions in Prostate Cancer: Implications for Pathological Practice. Mod Pathol. 2011;24(8):1128-1138.
6. Chaux A, Albadine R, Toubaji A, et al. Immunohistochemistry for ERG Expression as a Surrogate for TMPRSS2-ERG Fusion Detection in Prostatic Adenocarcinomas. Am J Surg Pathol. 2011;35(7):1014-1020.
7. Furusato B, Tan SH, Young D, 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;13(3):228-237.
8. Park K, Tomlins S, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 2010;12(7):590-598.
9. Falzarano SM, Zhou M, Carver P, et al. ERG Gene Rearrangement Status in Prostate Cancer Detected by Immunohistochemistry. Virchows Archiv. 2011;459(4):441-447.
10. Fisher KW, Zhang S, Wang M, et al. TMPRSS2-ERG Gene Fusion Is Rare Compared to PTEN Deletions in Stage T1a Prostate Cancer. Mol Carcinog. 2017;56(3):814-820.

11. Gopalan A, Leversha MA, Dudas ME, et al. TMPRSS2-ERG Rearrangement in Dominant Anterior Prostatic Tumours: Incidence and Correlation with ERG Immunohistochemistry. Histopathology. 2013;63(2):279-286.
12. Jiang H, Mao X, Huang X, et al. TMPRSS2:ERG Fusion Gene Occurs Less Frequently in Chinese Patients with Prostate Cancer. Tumor Biol. 2016;37(9):12397-12402.
13. Sung J-Y, Jeon HG, Jeong BC, et al. Correlation of ERG Immunohistochemistry with Molecular Detection of TMPRSS2-ERG Gene Fusion. J Clin Pathol. 2016;69(7):586-592.
14. Svensson MA, Perner S, Ohlson A-L, et al. A Comparative Study of ERG Status Assessment on DNA, mRNA, and Protein Levels Using Unique Samples from a Swedish Biopsy Cohort. Applied Immunohistochemistry and Molecular Morphology. 2014;22(2):136-141.
15. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
16. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
17. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
18. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
F	Updates to Specimen Preparation, Staining Procedure, Analytical Performance and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany
+800 5505 6606

CE 0123