anti-ERG (EPR3864) Rabbit Monoclonal Primary Antibody

REF 790-4576

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IVD Σ 50

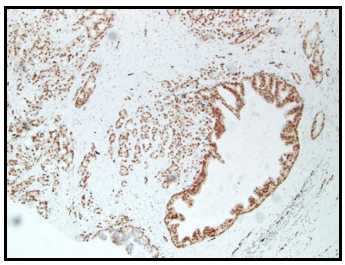

Figure 1. Prostate carcinoma positively staining with anti-ERG (EPR3864).

INTENDED USE

Anti-ERG (EPR3864) Rabbit Monoclonal Primary Antibody (anti-ERG (EPR3864)) is directed against the C-terminus of the ETS transcription regulator, ERG, and is capable of detecting both wildtype ERG, and truncated ERG resulting from ERG gene rearrangement. This antibody exhibits a nuclear staining pattern and may be used to aid in the identification of prostate adenocarcinomas through the detection of truncated ERG. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

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

Anti-ERG (EPR3864) is a rabbit monoclonal primary antibody produced against carboxy-terminal region of the ERG protein. As such, this antibody detects both wildtype and truncated ERG. Wildtype ERG is most notably expressed in vessel endothelium while truncated ERG is detected predominantly in prostate carcinomas that have undergone gene rearrangements with the 5' untranslated region of the androgen regulated TMPRSS2 gene (21q22.2-3).[1-5] Positive gene-fusion status of ERG has been indicated as an early marker of tumorogenesis in a subset of prostate carcinomas.[1-4] *In vitro* studies have shown ERG over-expression to be associated with cell migration, but lacking the ability to induce proliferative cell growth.[3] In one cohort, ERG positive gene-fusions were observed in 48.5% of clinically localized PCA, 30% of hormone naive metastases, 33% of hormone refractory metastases, and in 19% of high grade prostatic intraepithelial neoplasia lesions in intermingling to cancer foci. In the same cohort hyperplastic, atrophic, and benign prostate tissues were found to be negative for ERG gene-fusions.[2] The gene rearrangement status of the tissues reported in the literature was determined by FISH assays; however recent studies have shown a high correlation between FISH determined rearrangement status and protein over-expression detectable by IHC.[5]

REAGENT PROVIDED

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

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

The antibody is diluted in a TBS buffer containing 0.3% carrier protein.

Total protein concentration of the reagent is approximately 3.0 mg/mL. Specific antibody concentration is approximately 23 µg/mL. There is no known non-specific antibody reactivity observed in this product.

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

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General 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.

STORAGE

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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana BenchMark Series automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[6] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

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. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Avoid microbial contamination of reagents as it may cause incorrect results.
5. Consult local and/or state authorities with regard to recommended method of disposal.
6. Refer to the product Safety Data Sheet for additional information.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana BenchMark Series automated slide stainer in combination with Ventana detection kits and accessories. Refer to Table 1 and Table 2 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's Operator's Manual. Refer to the appropriate Ventana detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-ERG (EPR3864) with *ultra*View Universal DAB Detection Kit on a BenchMark XT and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Table 2. Recommended Staining Protocol for anti-ERG (EPR3864) with *i*VIEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

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".[7]

POSITIVE TISSUE CONTROL

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

STAINING INTERPRETATION

The cellular staining pattern for anti-ERG (EPR3864) 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) demonstrates a known cross-reactivity with the FLI-1 protein, which does not interfere with the analysis of prostate samples.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 3 and Table 4 and in the Repeatability section.

Specificity

Table 3. Specificity of anti-ERG (EPR3864) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/2	Stomach	0/3
Hypophysis	0/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	3/3	Kidney	0/3
Tonsil	1/3	Prostate	0/3
Endometrium	0/3	Cervix	0/3
Skeletal muscle	0/3	Skin	0/3
Nerve (sparse)	0/3	Mesothelium and lung	0/3

Sensitivity

Table 4. Sensitivity of anti-ERG (EPR3864) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma	0/1
Mucinous papillary adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma	0/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast intraductal carcinoma	0/1
Breast lobular carcinoma *in situ*	0/1
Breast invasive ductal carcinoma	0/1

Pathology	# positive / total cases
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Esophageal squamous cell carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinomas	0/1
Gastrointestinal adenocarcinoma	0/1
GIST	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	40/182
Prostatic transitional cell carcinoma	0/1
Prostatic Hyperplasia	0/21
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	0/1
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Diffuse malignant lymphoma	2/2
Hodgkin lymphoma	0/1
Diffuse malignant B-Cell lymphoma	0/1
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Malignant melanoma	0/1

Repeatability

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

- Inter-lot repeatability of the antibody.
- Intra-run and Inter-run repeatability on a BenchMark XT instrument.
- Intra-platform repeatability on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform repeatability between the BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

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3. Carver B, Tran J, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nature Genet. 2009;41(5):619-624.
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