NKX3.1 (RM)

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3189-091817

BIOCARE MEDICAL

Catalog Number:	**ACI 3189 A, B**	**API 3189 AA**
Description:	0.1, 0.5 ml, concentrated	6.0 ml, prediluted
Dilution:	1:50	Ready-to-use
Diluent:	Renoir Red	N/A

Intended Use:
For In Vitro Diagnostic Use
NKX3.1 (RM) [EP356] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of NKX3.1 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:
NKX3.1 is a protein found in humans and is encoded by the NKX3.1 gene located on chromosome 8 (1). The homeodomain containing transcription factor NKX3A is a putative prostate tumor suppressor that is expressed in a largely prostate-specific and androgen-regulated manner. NKX3.1 protein has been found to be positive in the vast majority of primary prostatic adenocarcinomas (1). A recent study showed that NKX3.1 staining was highly sensitive and specific for high-grade prostatic adenocarcinomas (2). The sensitivity for identifying metastatic prostatic adenocarcinomas overall was 98.6% (68/69 cases positive) for NKX3.1, and 94.2% (65/69 cores positive) for PSA (2). The specificity of NKX3.1 was 99.7% (1/349) in various cancers (2). NKX3.1 stains nuclei in both normal and prostate cancer; thus providing a robust stain that is easy-to-interpret, similar to other transcription factors such as TTF-1 or CDX2 (2).

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: Rabbit monoclonal
Species Reactivity: Human; others not tested
Clone: EP356
Isotype: IgG
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: NKX3.1
Immunogen: A synthetic peptide corresponding to residues of human NKX3.1 protein
Cellular Localization: Nuclear
Positive Tissue Control: Normal prostate or prostate cancer

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 (manual use):
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker 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 minutes at RT.
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a secondary-conjugated 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.

Protocol Recommendations (Ventana BenchMark ULTRA) using *ultra*View:
Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template: U ultraView DAB
Pretreatment Protocol: ULTRA CC1 Extended (92 min) at 95°C
Primary Antibody: Incubate for 32 minutes at 37°C
Detection: *ultra*View

Protocol Recommendations (Ventana BenchMark ULTRA) using OptiView:
Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template: U OptiView DAB IHC
Pretreatment Protocol: ULTRA CC1 32 minutes at 100°C
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: Incubate for 16 minutes at 36°C
Detection: OptiView

Technical Note:
This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

Performance Characteristics:
Sensitivity and specificity on diseased tissue and tissue cross-reactivity on normal tissue is summarized in Tables 1 and 2, respectively.

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, 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.

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Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

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 (NaN_3) 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) (3)
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. (4)
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.

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:

1. Bowen C, Gelmann EP. NKX3.1 activates cellular response to DNA damage. Cancer Res. 2010 Apr 15; 70(8):3089-97.
2. Gurel B, *et al.* NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol. 2010 Aug; 34(8):1097-105.
3. 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."
4. 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.

Produced using Abcam's RabMAb® technology. RabMAb® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.

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Table 1: Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Breast Cancer	0	10
Colon Cancer	0	19
Lung Cancer	0	11
Prostate Cancer	14	14

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebellum	0	1
Cerebral Cortex	0	1
Pituitary	0	1
Adrenal Gland	0	1
Thymus	0	1
Tonsil	0	1
Thyroid	0	1
Esophagus	0	1
Stomach	0	1
Small Intestine	0	1
Colon cancer	0	1
Pancreas	0	1
Spleen	0	1
Ovary	0	1
Fallopian Tube	0	1
Placenta	0	1
Kidney	0	1
Bladder	0	1
Urethra	0	1
Breast	0	1
Prostate	1	1
Testis	1	1
Myocardium	0	1
Smooth Muscle	0	1
Skeletal Muscle	0	1
Aorta	0	1
Lung	0	1
Skin	0	1
Liver	0	1

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