NKX3.1 [D2Y1A]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3260-060223

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3260 A, B	0.1, 0.5 mL	1:100	Van Gogh Yellow
Predilute	API 3260 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3260 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3260 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3260 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
NKX3.1 [D2Y1A] 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 located in chromosome 8p and encodes a homeodomain transcription factor whose expression is largely restricted to the prostate and controlled by the androgen hormone (1). Loss of function of NKX3.1 results in human prostrate carcinoma and prostatic intraepithelial neoplasia (PIN). NKX3.1 stains nuclei in both normal and prostate cancer providing a robust stain that is easy to interpret (2). Studies show that NKX3.1 is highly sensitive and specific for high-grade prostatic adenocarcinoma and highly sensitive for metastatic prostatic adenocarcinoma. The sensitivity for identifying metastatic prostatic adenocarcinomas overall was 98.6% (68/69 cases positive) for NKX3.1 compared to 94.2% (65/69 cores positive) for PSA. In the appropriate clinical setting, the addition of IHC staining for NKX3.1, along with other prostate-restricted markers, may help to definitively determine prostatic origin in poorly differentiated metastatic carcinomas (3).

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. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit monoclonal
Species Reactivity: Human, others not tested
Clone: D2Y1A
Isotype: IgG
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: NKX3.1
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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: N/A
Polymer: Incubate for 30 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 Warp Red.
Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):
OPAI3260 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: NKX3.1 [D2Y1A] Rb
Protocol Template (Description): Rb HRP Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR1, high pH; 101°C
Block Option: Buffer
Reagent Name, Time, Temp.: NKX3.1 [D2Y1A] Rb, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):
AVI3260 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre-Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3260 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Protocol Name: IHC Protocol F
Detection: Bond Polymer Refine
HIER: 10 min with ER2
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

Performance Characteristics:
Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

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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.

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) (4)
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. (5)
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. He WW, *et al.* A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer. Genomics. 1997; 43 (1):69–77.
2. Le Magnen C, *et al.* Cooperation of loss of NKX3.1 and inflammation in prostate cancer initiation. Dis Model Mech. 2018;11(11).
3. Gurel B, *et al.* NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol. 2010;34(8):1097-1105.
4. 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."
5. 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.

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

Tissue	Positive Cases	Total Cases
Breast Adenocarcinoma (IDC)	0	24
Colon Adenocarcinoma	0	40
Lung Adenocarcinoma	0	24
Lung Squamous Cell Carcinoma	0	24
Prostate Adenocarcinoma	39	52

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

Tissue	Positive Cases	Total Cases
Cerebrum	0	0
Cerebellum	0	0
Adrenal	0	0
Ovary	0	0
Pancreas	0	0
Parathyroid	0	0
Pituitary	0	0
Testis	3	3
Thyroid	0	0
Breast	0	0
Spleen	0	0
Tonsil	0	0
Thymus	0	0
Bone Marrow	0	0
Lung	0	0
Heart	0	0
Esophagus	0	0
Stomach	0	0
Small Intestine	0	0
Colon	0	0
Liver	0	0
Salivary Gland	0	0
Kidney	0	0
Prostate	10	10
Uterus	0	0
Cervix	0	0
Skeletal Muscle	0	0
Skin	0	0
Peripheral Nerve	0	0
Lining Cells	0	0

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