

# Prostein + PSA + NKX3.1

Prediluted Antibody Cocktail  
Control Number: 901-3166-111519

**BIOCARE**  
M E D I C A L

**Catalog Number:** API 3166 AA  
**Description:** 6.0 mL, RTU  
**Dilution:** Ready-to-use  
**Diluent:** N/A

## Intended Use:

For In Vitro Diagnostic Use

Prostein + PSA + NKX3.1 is a cocktail of mouse monoclonal and rabbit polyclonal antibodies that is intended for laboratory use in the qualitative identification of Prostein, PSA and NKX3.1 proteins 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:

The prostein gene encodes a 553-amino acid type IIIa plasma membrane protein with a cleavable signal peptide and 11 transmembrane-spanning regions (1). IHC analysis has demonstrated that the Prostein antibody (also known as P501S) was expressed in the normal prostate tissue and the vast majority of malignant prostatic tissues, regardless of grade and metastatic status (2). Prostein expression was not detected in thousands of representative normal and malignant non-prostatic tissue samples (2). Prostein has a perinuclear-like staining pattern, as expression is found in the Golgi complex of prostate cells. Compared to the PSA antibody, prostein was positive in 99% of metastatic prostate adenocarcinomas while 97% of cases were positive for PSA. No tumor was negative for both markers (3).

Prostate specific antigen (PSA) is a chymotrypsin-like serine protease (kallikrein family) produced by the prostate epithelium. Studies have shown that PSA is highly specific and is used to confirm prostatic acinar cell origin in primary and metastatic carcinomas, and to rule out non-prostatic carcinoma mimics (4-5).

NKX3.1 is a nuclear protein encoded by the NKX3-1 gene located on chromosome 8p. NKX3.1 protein has been found to be expressed in the vast majority of primary prostatic adenocarcinomas with 99.7% specificity. Contrary to earlier studies, NKX3.1 positive staining has now been shown to be highly sensitive and specific for high-grade prostatic adenocarcinomas vs. high-grade urothelial carcinomas. In addition, the sensitivity for identifying metastatic prostatic adenocarcinomas overall was 98.6% (68/69) for NKX3.1 and 94.2% (65/69) for PSA (6).

The International Society of Urological Pathology suggests the use of Prostein and NKX3.1 in addition to PSA, p63 and CK HMW as prostate markers (7). The CK HMW with p63 and AMACR are useful for atypical glands suspicious for adenocarcinoma of the prostate. GATA3 may be used along with PSA to differentially diagnose urothelial carcinoma (7).

Each antibody in this triple antibody cocktail can be morphologically identified in prostate cancers as PSA is a cytoplasmic stain, Prostein is present in the Golgi apparatus with perinuclear staining and NKX3.1 is a nuclear stain. Simultaneous stains with PSA, Prostein and NKX3.1 may greatly improve the detection rate and identification of a significant majority of prostatic metastases, especially poorly differentiated carcinomas of an unknown primary (7).

## 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 secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

## Reagent Provided:

Prostein + PSA + NKX3.1 is provided as a prediluted antibody cocktail of anti-Prostein, anti-PSA and anti-NKX3.1 antibodies in buffer with carrier protein and preservative.

Antibody	anti-Prostein	anti-PSA	anti-NKX3.1
Clone	10E3	ER-PR8	N/A
Source	Mouse Monoclonal	Mouse Monoclonal	Rabbit Polyclonal
Isotype	IgG2a/kappa	IgG1/kappa	N/A
Epitope/ Antigen	Prostein	PSA	NKX3.1
Cellular Localization	Perinuclear	Cytoplasmic	Nuclear
Staining	Brown (DAB)	Brown (DAB)	Brown (DAB)

## Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date.

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Species Reactivity:** Human; others not tested

**Positive Tissue Control:** Normal prostate or prostate cancer

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 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.

**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 Notes:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## 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

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### Limitations Cont'd:

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](http://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<sub>3</sub>) 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 in 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>.

### 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. Xu J, *et al.* Identification and characterization of prostein, a novel prostate-specific protein. *Cancer Res.* 2001 Feb 15; 61(4):1563-8.
2. Kalos M, *et al.* Prostein expression is highly restricted to normal and malignant prostate tissues. *Prostate.* 2004 Aug 1; 60(3):246-56.
3. Sheridan T, *et al.* The role of P501S and PSA in the diagnosis of metastatic adenocarcinoma of the prostate. *Am J Surg Pathol.* 2007 Sep; 31(9):1351-5.
4. Hameed O, Humphrey PA. Immunohistochemistry in diagnostic surgical pathology of the prostate. *Semin Diagn Pathol.* 2005 Feb; 22(1):88-104.
5. Kunju LP, *et al.* Prostate-specific antigen, high-molecular-weight cytokeratin (clone 34betaE12), and/or p63: an optimal immunohistochemical panel to distinguish poorly differentiated prostate adenocarcinoma from urothelial carcinoma. *Am J Clin Pathol.* 2006 May; 125(5):675-81.
6. 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.
7. Epstein JI, *et al.* Best practices recommendations in the application of immunohistochemistry in the prostate: report from the International Society of Urologic Pathology consensus conference. *Am J Surg Pathol.* 2014 Aug; 38(8):e6-e19.
8. 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."

### References Cont'd:

9. 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.