Diagnostic BioSystems

IHC Made Affordable

# PIN5 Cocktail

Rabbit anti-p40 + Mouse anti-Cytokeratin (HMW)
and Rabbit anti-p504S (AMACR)

PDR057

Document #: DS-0347-D
Effective Date: 12/18/2023

| Catalog # | Description |
|---|---|
| PDR057 | Ready to use antibody for use with Diagnostic BioSystems PolyVue™ Plus - Two Step Detection System |

**Intended Use**

For In Vitro Diagnostic Use.

This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy. Clinical interpretation of staining results should be accompanied by histological studies with proper controls. Patients' clinical histories and other relevant diagnostic tests should be utilized by a qualified person(s) when evaluating and interpreting results.

**Components**

**Reagent A:** A mixture of rabbit anti-p40 (Polyclonal) and mouse anti-cytokeratin, high molecular weight (HMW) (clone 34βE12) antibodies.

**Reagent B:** Rabbit anti-p504S (alpha-Methylacyl-CoA Racemase (AMACR) antibody.

**Description**

**Reagent A:**

**Polyclonal rabbit antibody to human p40.** Anti-p40 is a polyclonal rabbit antibody, produced by Amino acids 5-17 (ENNAQTQFSEPQY) of human p40 (isoform of p63 delta), was used as immunogen for this antibody. The isotype is Rabbit IgG. This antibody is highly expressed in the basal or progenitor layers of many epithelial tissues.

**Monoclonal mouse antibody to human cytokeratin (HMW).** The anti-cytokeratin (HMW) is a monoclonal mouse antibody, clone 34βE12, produced by immunization with solubilized keratin extract from human stratum corneum. The isotype is IgG1, kappa. This antibody recognizes keratin polypeptides of 68, 58, 56.5 and 50 kD in stratum corneum extracts. The antibody reacts with squamous, ductal and other complex epithelia.

**Reagent B:**

**Rabbit anti-p504S (AMACR) antibody.** Anti-p504S (AMACR) is a polyclonal rabbit antibody, produced by immunization with synthetic human AMACR peptide. The antibody comes from a purified immunoglobulin fraction of rabbit antiserum against AMACR. P504S (AMACR) encodes a protein involved in the beta-oxidation of branched chain fatty acids.

**Summary and Explanation**

The combined detection of p504S, p40 and high molecular weight cytokeratin markers has been shown to be useful for distinguishing benign conditions mimicking cancer from prostate carcinomas. In particular, these markers have been shown to be relevant in diagnosing the premalignant condition, prostatic intraepithelial neoplasia (PIN).

High molecular weight cytokeratin and p63 are commonly used markers of basal epithelial cells. Benign prostate tissue contains basal cells, which are absent in prostate cancers. As p40 is an isoform of p63, it also stains basal epithelial cells and can be used in prostate cancer tissue.

P504S has been recently described as a prostate cancer-specific gene. Expression of p504S (AMACR) protein is seen in prostatic adenocarcinoma but not in benign prostatic tissue. Anti-p540S stains premalignant lesions of prostate: high-grade PIN and atypical adenomatous hyperplasia.

The PIN5 antibody combination may be particularly useful for confirming the diagnosis of prostate carcinoma in small foci of needle biopsies. The positive prostate cancer marker, p504S (AMACR), in conjunction with the negative basal cell markers (p40 and HMW cytokeratin) offers utility in diagnosing PIN in difficult cases where tissue may be limited.

**Format**

These antibody reagents have been pre-tittered and are ready-to-use in two-color, double sequential staining assays. The antibody solutions contain sodium azide and Proclin 300 as preservatives. The solutions are thimerosol-free.

**Principles of the Procedures**

Antigen detection by immunohistochemistry (IHC) is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary antibody may be used in IHC using manual techniques or using Diagnostic BioSystems Automated Montage 360™ Staining System.

**Staining Protocol**

Suggested Reagent A incubation period of 15 minutes and Reagent B for 30 minutes at room temperature. Optimal incubation time and conditions should be determined by the user based upon the fixation conditions and the staining system employed. Formalin fixed paraffin embedded tissue sections require high temperature antigen unmasking with 10 mM EDTA buffer, pH 8.0 prior to immunostaining.

**Dilution of Primary Antibody**

Diagnostic BioSystems ready to use antibodies have been optimized for use with the recommended Diagnostic BioSystems Detection System and do not require further dilution. Further dilution may result in loss of sensitivity. The user must validate any such change.

Diagnostic BioSystems concentrated antibodies must be diluted in accordance with the staining procedure when used with the recommended Diagnostic BioSystems detection system. Use of any detection methods other than the recommended systems and protocols require validation by the user. Antibody dilutions should be appropriately adjusted and verified according to the detection system used.

**Materials Required But Not Provided**

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com

**Storage and Handling**

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Store at 2-8°C. This antibody is suitable for use until the expiration date when stored at 2-8°C. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly. Unused portions of antibody preparation should be discarded after one day.

The presence of precipitate or an unusual odor indicates that the antibody is deteriorating and should not be used.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

**Specimen Collection and Preparation**

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

**Precautions**

This product is a single-use, non-sterile, in vitro diagnostic device.

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 (NaN3) 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). 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. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

**Treatment of Tissues Prior to Staining**

Place the slides in the recommended antigen retrieval solution using Diagnostic BioSystems Montage Opus™ Antigen Retrieval System. Allow slides to cool down for 20 minutes prior to staining.

**Staining Procedure**

Refer to the following table for conditions specifically recommended for this antibody. Refer to the Diagnostic BioSystems PolyVue™ Plus—Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for guidance on specific staining protocols or other requirements.

| Parameter | Diagnostic BioSystems Recommendations |
|---|---|
| Positive Control | Prostatic intraepithelial neoplasia (PIN) |
| Concentrated Dilution | Prediluted |
| Pretreatment | EDTA Buffer pH 8.0 |
| Incubation Time & Temperature | Reagent A: 15min@RT/ Reagent B:30 min @ RT |
| Detection System | PolyVue™ Plus - Two Step Detection System or Montage PolyVue Plus™ Auto Detection System for Montage 360 System |
| Tissue Type | FFPE |

**Quality Control**

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

**Troubleshooting**

Contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2, techsupport@dbiosys.com or your local distributor to report unusual staining.

**Cellular Localization**

p40: Nuclear

Cytokeratin (HMW): Cytoplasmic

P504S (AMACR): Cytoplasmic

**Limitations of the Procedure**

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B surface Antigen (HBsAg) may give a false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

**Performance Characteristics**

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, and 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 Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

**References**

i) Beach et al. Am J Surg Pathol 26 (12): 1588, 2002.
ii) Luo et al. Cancer Res 62 (8): 2220, 2002.
iii) Molinie et al. Mod Pathol 17: 1180, 2004.
iv) Tacha and Miller Appl Immunohistochem Mol Morphol 12 (1): 75, 2004.
v) Signoretti et al. Am J Pathol 157 (6): 1769,

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