BIOCARE MEDICAL

Cytokeratin 5/14 Cocktail, 2X

Prediluted Monoclonal Antibody Cocktail

Control Number: 901-3026-082614

ISO 9001&13485 CERTIFIED

Catalog Number: **API 3026 AAK**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use.
Cytokeratin 5/14 Cocktail, 2X is intended for laboratory use in the qualitative identification of cytoplasmic keratins CK5 and CK14 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:

CK5 [XM26] has been shown to have positive reactivity for CK5 protein and lack of reactivity for CK6 protein by ELISA. CK5 is distributed in many non-keratinizing stratified squamous epithelia such as tongue mucosa, basal epithelia, hair follicles, and trachea, as well as basal cells in prostate glands and myoepithelial cells in mammary glands. CK5 is also expressed in most epithelial and biphasic mesotheliomas. CK5 has been noted in the majority of lung squamous cell carcinoma and is expressed in luminal Type B breast cancers (triple negative).

CK14 is a human intermediate filament protein of 50 kDa. CK14 can be used to distinguish stratified epithelial cells from simple epithelial cells. It is expressed in basal epithelium in prostate and myoepithelium in normal breast. CK14 is a useful marker in the differential diagnosis of squamous cell carcinoma with poor clinical outcome. CK14 is also expressed in luminal Type B breast cancers, similar to CK5.

The CK5/CK14 monoclonal antibodies have been shown to be superior to CK5/6 and 34betaE12. Cytokeratin 5/14 Cocktail, 2X may be used to identify basal cells in prostate and myoepithelium cells in breast cancer. Sporadic loss of CK5/14 epithelium staining, along with p63, typically occurs in prostatic intraepithelial neoplasia (PIN), with nearly complete loss in prostate cancer. Additionally, CK5/CK14 + AMACR (P504S) may be added to the panel of antibodies used to assess neoplasia in prostate biopsies. A cocktail comprised of CK5/CK14 + p63 + P504S has become the standard care for PIN and prostate cancer diagnosis in many histopathology laboratories. Additionally, studies have shown that CK5/14-positive sporadic breast cancers arise from glandularly committed progenitor cells and represent about 9% of sporadic invasive ductal breast cancers and 78% of *BRCA1*-associated tumors.

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.

Source: Mouse monoclonal (CK5) and Mouse monoclonal (CK14)

Species Reactivity: Human; others not tested

Clone: XM26 (CK5) + LL002 (CK14)

Isotype: IgG1, kappa (CK5) + IgG3 (CK14)

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration

Epitope/Antigen: CK5 + CK14

Cellular Localization: Cytoplasmic

Positive Control: Normal prostate

Known Applications: Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Dropper Bottle (DB3026)

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:

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 product datasheet 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: Incubate for 10 minutes at RT with a probe.

Polymer: Incubate for 10 minutes at RT with a polymer.

Chromogen: Incubate for 5 minutes at RT when using Biocare's DAB – OR – Incubate for 5-7 minutes at RT when using Biocare's Warp Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

CK5/14, 2X has been tested with p63-2X (PM366), P504S 2X (PP365), AMACR, 2X (APA3016), and ERG, 2X (API3017) mixed 1:1, using Biocare's MACH 2 Double Stain 2. It can be used on an automated staining platform. Use TBS buffer 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 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.

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)
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.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.

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Precautions Cont'd:

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 MSDS is available upon request and is located at http://biocare.net/support/msds/.

References:

1. Abrahams NA, *et al.* Validation of cytokeratin 5/6 as an effective substitute for keratin 903 in the differentiation of benign from malignant glands in prostate needle biopsies. Histopathology. 2002 Jul;41(1):35-41.
2. Shah RB, *et al.* Comparison of the basal cell-specific markers, 34betaE12 and p63, in the diagnosis of prostate cancer. Am J Surg Pathol. 2002 Sep;26(9):1161-8.
3. Bhargava R, *et al.* CK5 is more sensitive than CK5/6 in identifying the "basal-like" phenotype of breast carcinoma. Am J Clin Pathol. 2008 Nov;130(5):724-30.
4. Reis-Filho JS, *et al*. Distribution of p63, cytokeratins 5/6 and cytokeratin 14 in 51 normal and 400 neoplastic human tissue samples using TARP-4 multi-tumor tissue microarray. Virchows Arch. 2003 Aug;443(2):122-32.
5. Laakso M, *et al.* Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. Mod Pathol. 2005 Oct;18(10):1321-8.
6. 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."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA 2005.

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.

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