Cytokeratin 5/14 Cocktail

Concentrated and Prediluted Monoclonal Antibodies
901-3025-040819

BIOCARE MEDICAL

Catalog Number:	**ACI 3025 A, C**	**API 3025 AA**	**OAI 3025 T60**	**VLTM 3025 G20**
Description:	0.1, 1.0 mL, conc.	6.0 mL, RTU	60 tests, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Renaissance Background Reducing	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
Cytokeratin 5/14 Cocktail [XM26 + LL002] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of cytokeratin proteins 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, 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 also been noted in large cell carcinomas and pulmonary squamous cell carcinoma. CK5 is expressed in luminal Type B breast cancers (triple negative).
CK14 is a human intermediate filament protein of 50kDa. 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 betaE12. Cytokeratin 5/14 Cocktail may be used to identify basal cells in prostate and myoepithelium cells in breast cancer. Loss of epithelium staining along with p63 typically occurs in PIN (prostatic intraepithelial neoplasia) and prostate cancer. Additionally, CK5/ CK14 + AMACR (P504S) may be added to the panel of antibodies used to assess neoplasia in prostate biopsies. The cocktail comprised of CK5 + CK14 + p63 + P504S has become the standard care for PIN and prostate cancer diagnosis in many histopathology laboratories. 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 one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: XM26 + LL002
Isotype: IgG1/kappa + IgG3
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: CK5 + CK14
Cellular Localization: Cytoplasmic
Positive Tissue Control: Normal prostate
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 (VALENT® Automated Slide Staining Platform):
VLTM3025 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: Incubate for 10 minutes with Val Mouse Secondary.
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 10 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva or Reveal Decloaker. Refer to the Diva or Reveal Decloaker product data sheet for specific instructions.
Protein Block (**Optional):** Incubate for 5-10 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 Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

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Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI3025 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: CK5/14
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR2, low pH; 103°C
Reagent Name, Time, Temp.: CK5/14, 30 min., 25°C

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 an 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) (6)
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. (7)
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. 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.

References Cont'd:

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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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