CK5/14 + p63 + CK7/18

Prediluted Multiplex Antibody Reagent
901-360DS-040719

BIOCARE MEDICAL

Catalog Number:	**PM 360 DS AA, H**	**OAI 360 T60**	**VLTMR 360 G20**
Description:	6.0, 25 mL, RTU	60 tests, RTU	20 mL, RTU
Dilution:	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
CK5/14 + p63 + CK7/18 is intended for laboratory use in the qualitative identification of keratins CK5, CK7, CK14, and CK18, and p63 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:
CK5/14 + p63 + CK7/18 is comprised of mouse monoclonal anti-CK5, anti-CK14, and anti-p63 antibodies and rabbit monoclonal anti-CK7 and anti-CK18 antibodies. CK5 and CK14 are high molecular weight keratins expressed in the cytoplasm of basal cells and myoepithelium of breast tissue (1-4). p63 is a transcription factor present in the nuclei of myoepithelial cells (2,4). In contrast, CK7 and CK18 are low molecular weight cytokeratins primarily expressed in luminal cells of the breast (1-3).

CK5, CK14, p63, CK7 and CK18 have routinely been used as IHC markers to complement morphological evaluation in the assessment of breast lesions, due to the differential expression of the luminal vs. basal and myoepithelial markers (1-5). Cases of usual ductal hyperplasia (UDH) have been associated with expression of the basal cell markers, intermixed with cells expressing the keratins of luminal cells (1-2,6-10). Most cases of atypical ductal hyperplasia (ADH) and low grade ductal carcinoma *in situ* (LG-DCIS) were negative for the basal markers and exhibited an immunophenotype indicative of luminal cells (1,5-8). Additionally, the basal phenotype has been shown to be characterized by luminal expression of the basal and myoepithelial markers, using a cocktail of CK5, CK14 and p63 (11-13).

IHC, using CK5, CK14, p63, CK7 and CK18 antibodies, evaluated in combination with hematoxylin and eosin (H&E), has been shown to significantly increase inter-observer agreement amongst pathologists, compared to H&E alone (14).

Principle of Procedure:
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:

CK5/14 + p63 + CK7/18 is provided as a prediluted antibody cocktail of anti-CK5, anti-CK14, anti-p63, anti-CK7 and anti-CK18 antibodies, in buffer with carrier protein and preservative.

Antibody	anti-CK5	anti-CK14	anti-p63	anti-CK7	anti-CK18
Clone	XM26	LL002	4A4	BC1	EP30*
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG3	IgG2a/kappa	IgG	IgG
Epitope/ Antigen	CK5	CK14	p63	CK7	CK18
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear	Cytoplasmic	Cytoplasmic
Staining	Brown (DAB)	Brown (DAB)	Brown (DAB)	Red	Red

*Previously known as E431-1

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
Positive Tissue Control: Breast carcinoma

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTMR360 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: Incubate for 10 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Double Stain Detection: Incubate for 30 minutes using Val Plex 2.
Chromogen (1): Incubate for 5 minutes with Val DAB.
Chromogen (2): Incubate for 15 minutes with Val Fast Red.
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 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 30-60 minutes at RT.
Double Stain Detection: Incubate for 30 minutes at RT using MACH 2 Double Stain 2.
Chromogen (1): Incubate for 5 minutes at RT with Betazoid DAB.
Chromogen (2): Incubate for 5-7 minutes at RT with Warp Red. Rinse with deionized water.

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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 optimized for use with MACH 2 Double Stain 2. Use TBS buffer for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):
OAI360 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 p63 7/18
Protocol Template (Description): Multiplex 2 Template 1
Dewaxing (DS Option): DS Buffer
Antigen Retrieval (AR Option): AR2, low pH; 101°C
Reagent Name, Time, Temp.: CK5/14 p63 7/18, 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 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) (15)
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. (16)
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:

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8. Boecker W, *et al.* Usual ductal hyperplasia of the breast is a committed stem (progenitor) cell lesion distinct from atypical ductal hyperplasia and ductal carcinoma in situ. J Pathol. 2002; 198:458-67.
9. Koo JS, *et al.* Comparison of Immunohistochemical Staining in Breast Papillary Neoplasm of Cytokeratin 5/6 and p63 in core Needle Biopsies and Surgical Excisions. Appl Immunohistochem Mol Morph. 2012; 20:108-15.
10. Ichihara S, *et al.* Double immunostaining with p63 and high-molecular-weight cytokeratins distinguishes borderline papillary lesions of the breast. Path Int. 2007; 57:126-32.
11. Livasy CA, *et al.* Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 2006; 19:264-71.
12. Laakso M, *et al.* Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. Mod Pathol. 2005; 18:1321-8.
13. Bhargava R, *et al.* CK5 is More Sensitive than CK5/6 in Identifying the "Basallike" Phenotype of Breast Carcinoma. Am J Clin Pathol. 2008; 130:724-30.
14. Jain RK, *et al.* Atypical ductal hyperplasia: interobserver and intraobserver variability. Mod Pathol. 2011; 24:917-23.
15. 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."
16. 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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