

CK5/14 + p63 + CK7/18
Prediluted Multiplex Antibody Reagent
Control Number: 902-360DS-090517

Catalog Number: APR 360DS AA, H
Description: 6.0, 25 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

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 Warp 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 (Warp Red)	Red (Warp Red)

*Previously known as E431-1

Storage and Stability:

Store at 2°C to 8°C. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues).

Species Reactivity: Human

Positive Tissue Control: Breast cancer

Staining Protocol Recommendations:

Deparaffinization and rehydration: Perform deparaffinization of tissues with xylenes or xylene substitute, followed by rehydration through graded alcohols.

Peroxide Block: Block for 5 minutes with Biocare's Peroxidized 1.

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

Protein Block: Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30-60 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

Chromogen (1): Incubate for 5 minutes at RT with Biocare's Betazoid DAB.

Chromogen (2): Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

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

Technical Notes:

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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 (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) (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 in 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>.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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References:

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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.
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11. Livasy CA, *et al.* Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol.* 2006; 19:264-71.
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