Breast Cocktail (CK HMW/p63 + CK7/8/18)

Prediluted Multiplex Antibody Reagent
902-3203DSK-05172021

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
UltraLine – For BenchMark	AVR 3203DSK G, G25	6.0, 25mL	Ready-to-use	N/A

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

Summary and Explanation:
Breast Cocktail (CK HMW/p63 + CK7/8/18) is comprised of mouse monoclonal anti-CK HMW and anti-p63 antibodies as well as rabbit monoclonal anti-CK7 and mouse monoclonal anti-CK8/18 antibodies. CK HMW (high molecular weight cytokeratin) is 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, CK8 and CK18 are low molecular weight cytokeratins primarily expressed in luminal cells of the breast (1-3).
CK HMW, p63, CK7, CK8 and CK18 have routinely been used as a panel of 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 negativefor the basal markers and exhibited an immunophenotype indicative ofluminal 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 CK HMW and p63 (11-13).
IHC, using CK HMW, p63, CK7, CK8 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:
Breast Cocktail (CK HMW/p63 + CK7/8/18) is provided as follows:

1. Prediluted antibody cocktail of anti-CK HMW and anti-p63 antibodies(AVR3204G), in buffer with carrier protein and preservative.

Antibody	anti-CK HMW	anti-p63
Clone	34βE12	4A4
Source	Mouse monoclonal	Mouse monoclonal
Isotype	IgG1/kappa	IgG2a/kappa
Epitope/ Antigen	CK HMW	p63
Cellular Localization	Cytoplasmic	Nuclear
Staining	DAB	DAB

Reagents Provided Cont'd:
2. Prediluted antibody cocktail of anti-CK7, anti-CK8/18 antibodies(AVR3205G), in buffer with carrier protein and preservative.

Antibody	anti-CK7	anti-CK8/18
Clone	BC1	5D3
Source	Rabbit monoclonal	Mouse monoclonal
Isotype	IgG	IgG1
Epitope/ Antigen	CK7	CK8/18
Cellular Localization	Cytoplasmic	Cytoplasmic
Staining	Red	Red

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.

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

Species Reactivity: Human

Positive Tissue Control: Breast Cancer

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
Refer to the User Manual for specific instructions for use.Recommended protocol parameters are as follows:
Template: U IHC DS uDAB-uRed Template
Pretreatment Protocol: ULTRA CC1 Standard (64 min) at 95°C
Primary Antibody (AVR3204): Incubate for 32 minutes at 37°C
Denaturation: Default Template setting (4 minutes at 90°C)
Primary Antibody (AVR3205): Incubate for 32 minutes at 37°C
ultraBlock (V-Blocker BRI4001): Incubate for 4 minutes (with appropriate Option # registered by user) V-Blocker is highly recommended to be applied prior to any detection system.
Detection: ultraView DAB and AP Detections

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 (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)

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Precautions Cont'd:

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.

Technical Support:

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

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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13. 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; 130:724-30.
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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."

References Cont'd:

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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