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

Prediluted Multiplex Antibody Cocktail
901-3203DSK-100417

BIOCARE MEDICAL

VP Echelon™ Series

Catalog Number: **AVI 3203DSK G**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Intended Use:

For In Vitro Diagnostic Use

Breast Cocktail (CK HMW/p63 + CK7/8/18) is a cocktail of mouse and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of high molecular weight cytokeratin (CK1, 5, 10, 14), keratins CK7, CK8, CK18, and p63 proteins 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:

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 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 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:

A sequential double stain is used for the simultaneous detection of two different antigens within one tissue section. A primary antibody is applied to the tissue, followed by a horseradish peroxidase (HRP) detection system. A denaturing step is required to eliminate cross-reactivity from the application of the second detection system. A second primary antibody is then applied, followed by an alkaline phosphatase (AP) detection system. Visualization of antigens is achieved with DAB and Red chromogens.

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 (AVI3204G), 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

2. Prediluted antibody cocktail of anti-CK7, anti-CK8/18 antibodies (AVI3205G), 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. 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.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human

Positive Tissue Control: Breast cancer

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 (AVI3204): Incubate for 32 minutes at 37°C
Denaturation: Default Template setting (4 minutes at 90°C)
Primary Antibody (AVI3205): 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: *ultra*View DAB and AP Detections

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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) (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 bymouth 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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4. Lerwill MF. Current practical applications of diagnostic immunohistochemistry in breast pathology. Am J Surg Pathol. 2004; 28:1076-91.

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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.
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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.
12. Laakso M, *et al.* Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. Mod Pathol. 2005; 18:1321-8.
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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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