



## ADH-5 Breast Marker Antibody Cocktail

Prediluted Multiplex Antibody Reagent

Control Number: 901-360-052212

ISO  
9001&13485  
CERTIFIED

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

**Intended Use:**

For In Vitro Diagnostic Use.

ADH-5™ Breast Marker Antibody Cocktail is intended for laboratory use in the qualitative identification of cytoplasmic keratins CK5, CK7, CK14, and CK18, and the nuclear marker p63 by immunohistochemistry (IHC) in formalin fixed paraffin embedded (FFPE) human tissues. ADH-5 may aid pathologists in evaluating the histologic subtype of breast lesions, following a primary diagnosis using hematoxylin and eosin. 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:**

ADH-5™ Breast Marker Antibody Cocktail 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 difficult to diagnose 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). In contrast, 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 a cocktail of CK5, CK14, p63, CK7 and CK18 antibodies, evaluated in combination with hematoxylin and eosin (H&E), has been shown to significantly increase diagnostic inter-observer agreement amongst pathologists, compared to H&E alone (14).

**Principle of Procedure:**

ADH-5 Breast Marker Antibody Cocktail is a primary antibody cocktail of mouse monoclonal and rabbit monoclonal 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 sequential application of chromogenic substrates (e.g. DAB and Fast 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. 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:**

ADH-5 Breast Marker Antibody Cocktail 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	BC4A4	BC1	E431-1
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal	Rabbit monoclonal
Isotype	IgG1/kappa	IgG3	IgG2a/kappa	Rabbit IgG	Rabbit 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)

**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 carcinoma

**Negative Tissue Control:** Lymph node

**Protocol Recommendations:**

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

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1 (PX968).

**Pretreatment:** Perform heat retrieval using Biocare's Diva Decloaker (DV2004 or DV2004X). Refer to the Diva Decloaker product datasheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with Biocare's Background Punisher (BP974).

**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 (MRCT525).

**Chromogen (1):** Incubate for 5 minutes at RT with Biocare's Betazoid DAB (BDB2004).

**Chromogen (2):** Incubate for 5-7 minutes at RT with Biocare's Warp Red (WR806). Rinse in deionized water.

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

**Technical Notes:**

This antibody cocktail can also be used on an automated staining system. Use TBS buffer for washing steps.

**Limitations:**

The optimum 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 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.





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### 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<sub>3</sub>) 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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

### 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.

### Limitations and Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

### References:

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3. Yeh IT, Mies C, Application of Immunohistochemistry to Breast Lesions. *Arch Pathol Lab Med* 2008; 132:349-57.
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5. Moriya T, et al. Usefulness of immunohistochemistry for differential diagnosis between benign and malignant breast lesions. *Breast Cancer* 2009; 16:173-178.
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10. Ichihara S, et al. Double immunostaining with p63 and high-molecular-weight cytokeratins distinguishes borderline papillary lesions of the breast. *Path Int* 2007; 57:126-132.
11. Livasy CA et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 2006; 19:264-271.
12. Laakso M, Loman N, Borg A, Isola J. 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 "Basal-like" Phenotype of Breast Carcinoma. *Am J Clin Pathol* 2008; 130:724-730.
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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."
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