



ADH-5 (Breast Marker Cocktail)

Prediluted Double Stain Antibody (4-Step)

Control Number: 901-360DS-122208

ISO
9001:2000
CERTIFIED

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

Intended Use:
For In Vitro Diagnostic Use

Summary and Explanation:

ADH-5 Breast Marker Cocktail (Atypical Ductal Hyperplasia) is composed of CK5+CK14+p63+CK7+CK18 antibodies. This cocktail can be used in a wide range of applications for breast cancer. Invasive versus noninvasive breast lesions can be easily distinguished with the presence or absence of myoepithelium (CK5/14 and/or p63) (DAB) and glandular staining of breast cancer with CK7/18 (Fast Red). This 5 antibody cocktail in one single application can also distinguish hyperplasia of the usual type versus atypical hyperplasia, and distinguish micro invasive and basal phenotypes in most breast cancers.

Luminal or cytoplasmic staining may also be observed in CK5/14 and/or CK7/18 staining (bimodal). In certain cases, only CK5/14 luminal staining is observed representing a basal-phenotype classification. Breast cancer with bimodal and/or basal-like staining are associated with poor prognosis.

Principle of Procedure:

Antigen detection, in tissues and cells, is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigens with their respective primary antibodies, a secondary double stain detection (double label) is added to bind the primary antibodies. This detection is evidenced by a colorimetric reaction.

Source: Mouse and Rabbit monoclonal

Species Reactivity: Human

Clone: XM26, LL002, BC4A4, BC1 and E431-1

Isotype: IgG1/kappa, IgG3, IgG2a/kappa, Rabbit IgG and Rabbit IgG

Epitope/Antigen: CK5/14 + p63 + CK7 + CK18

Cellular Localization:

CK5+CK14: cytoplasmic (brown), p63: nuclear (brown), CK7+CK18: cytoplasmic

Positive Control: Breast Cancer

Normal Tissue: Normal breast and normal prostate

Abnormal Tissue: Breast cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative.

Storage and Stability:

Store at 2°C to 8°C. 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Peroxide Block:

Block for 5 minutes with BIOCARE's PEROXIDAZED 1.

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure at 95°C for 40 minutes using BIOCARE's Decloaking Chamber, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 20 minutes then wash in distilled water.

Protein Block:

Optional: Incubate for 15-30 minutes at RT with BIOCARE's Background Sniper.

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

Double Stain Detection:

Incubate for 30 minutes at RT.

Chromogen (1): Incubate for 5 minutes at RT when using BIOCARE's Betazoid DAB.

Chromogen (2):

Incubate for 10 minutes at RT with BIOCARE's Vulcan Fast Red. Rinse in deionized water.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

Technical Note:

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

Performance Characteristics:

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information about Tissue Controls.

Precautions:

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)

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.

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

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.





ADH-5 (Breast Marker Cocktail)

Prediluted Double Stain Antibody (4-Step)

Control Number: 901-360DS-122208

ISO
9001:2000
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References:

1. Boecker W, Moll R 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 Dec;198(4):458-67
2. Laakso M, Loman N, Borg A, Isola J. Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. *Mod Pathol.* 2005 Oct;18(10):1321-8. Links
3. Lacroix-Triki M, Mery E, Voigt JJ, Istier L, Rochaix P. Value of cytokeratin 5/6 immunostaining using D5/16 B4 antibody in the spectrum of proliferative intraepithelial lesions of the breast. A comparative study with 34betaE12 antibody. *Virchows Arch.* 2003 Jun;442(6):548-54. Epub 2003 Apr 24. Links
4. 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."
5. National Committee for Clinical Laboratory Standards(NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.



ADH-5 (Breast Marker Cocktail)

Prediluted Mouse Monoclonal and Rabbit Monoclonal Double Stain Antibody (4-Step)

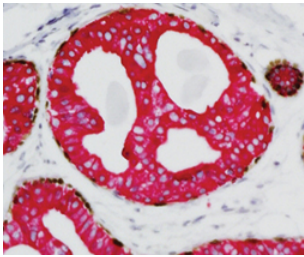
Control Number: 901-360DS-122908

Catalog Number: IP 360DS G10
Description: 10 ml, predilute

Intended Use:
For In Vitro Diagnostic Use

Summary and Explanation:
ADH-5 Breast Marker Cocktail (Atypical Ductal Hyperplasia) is composed of CK5+CK14+p63+CK7+CK18 antibodies. This cocktail can be used in a wide range of applications for breast cancer. Invasive versus noninvasive breast lesions can be easily distinguished with the presence or absence of myoepithelium (CK5/14 and/or p63) (DAB) and glandular staining of breast cancer with CK7/18 (Fast Red). This 5 antibody cocktail in one single application can also distinguish hyperplasia of the usual type versus atypical hyperplasia, and distinguish micro invasive and basal phenotypes in most breast cancers.

Luminal or cytoplasmic staining may also be observed in CK5/14 and/or CK7/18 staining (bimodal). In certain cases, only CK5/14 luminal staining is observed representing a basal-phenotype classification. Breast cancer with bimodal and/or basal-like staining are associated with poor prognosis.



Hyperplasia of the usual type; p63 staining myoepithelia, CK5/14 and CK7/18, luminal.

Source: Mouse and Rabbit monoclonal

Species Reactivity: Human

Clone: XM26, LL002, BC4A4, BC1 and E431-1

Isotype: IgG1/kappa, IgG3,

Antibody Category: Breast carcinoma

Epitope/Antigen: CK5/14 + p63 + CK7 + CK18

Cellular Localization:

CK5+CK14: cytoplasmic (brown), p63: nuclear (brown), CK7+CK18: cytoplasmic (red)

Positive Control: Breast cancer

Normal Tissue: Normal breast and normal prostate

Abnormal Tissue: Breast cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative.

Storage and Stability:

Store at 2°C to 8°C. 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure using BIOCARE's Decloaking Chamber at 95°C for 40 minutes, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 20 minutes then wash in distilled water.

Peroxide Block:

Block for 5 minutes at RT.

Primary Antibody:

Incubate for 30 minutes at RT.

Double Stain Detection:

Incubate for 30 minutes at RT using BIOCARE's Multiplex Stain Kit 2.

Chromogen (1):

Incubate for 5 minutes at RT when using BIOCARE's DAB.

Chromogen (2):

Incubate for 20 minutes at RT with BIOCARE's Fast Red. Rinse in deionized water.

Counterstain:

Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

Quality Statement:

BIOCARE protocols have been standardized using in-house antibodies, detection and accessory reagents for use on the *intelliPATH* FLX automated stainer. Recommended staining protocols are specified in the datasheet of the antibody of interest. Pre-optimized *intelliPATH* FLX protocols with preset parameters can be displayed, printed and edited according to the procedure in the operator's manual. Refer to the operator's manual for additional instruction to navigate *intelliPATH* FLX software and stainer. Use TBS for washing steps unless otherwise specified.

Performance Characteristics:

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

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Precautions:

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ADH-5 (Breast Marker Cocktail)

Prediluted Mouse Monoclonal and Rabbit Monoclonal Double Stain Antibody (4-Step)

Control Number: 901-360DS-122908

Precautions cont'd:

Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

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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2. Laakso M, Loman N, Borg A, Isola J. Cytokeratin 5/14-positive breast cancer: true basal phenotype confined to BRCA1 tumors. *Mod Pathol.* 2005 Oct;18(10):1321-8. [Links](#)
3. Lacroix-Triki M, Mery E, Voigt JJ, Istier L, Rochoix P. Value of cytokeratin 5/6 immunostaining using D5/16 B4 antibody in the spectrum of proliferative intraepithelial lesions of the breast. A comparative study with 34betaE12 antibody. *Virchows Arch.* 2003 Jun;442(6):548-54. [Epub 2003 Apr 24. Links](#)
4. 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."
5. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.

