CDX2 (M) + CDH17 (RM)

Prediluted Multiplex Antibody Reagent
901-3135DS-090117

BIOCARE MEDICAL

Catalog Number: **API 3135DS AA**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use
CDX2 (M) + CDH17 (RM) is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of CDX2 and CDH17 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:
CDX2 is a homeobox gene that encodes an intestine-specific transcription factor (1). CDX2 has been useful to establish gastrointestinal origin of metastatic adenocarcinomas and carcinoids and can be especially useful in distinguishing metastatic colorectal adenocarcinoma from tumors of unknown origin (1-7). CDX2 has been shown to be more specific and more sensitive than villin or CK20 (1,4,6). CDX2 has also been shown to be expressed in mucinous ovarian cancer, bladder adenocarcinoma, cholangiocarcinoma and malignant germ cell tumors of the testes (1,2,6-8). Only very rare examples of carcinomas of the genitourinary and gynecologic tracts or breast, lung, and head and neck cancers showed elevated levels of CDX2 expression (1).
CDH17 (Cadherin 17 or LI-cadherin) is a novel oncogene expressed in intestinal epithelium which is involved in tumor invasion and metastasis (9-10). CDH17 is a highly specific marker in colon cancer (99/99, 100%) and is a more sensitive marker than CDX2 (93/99, 94%) and CK20 (91/99, 92%) (11). Overexpression of CDH17 (and conversely, under expression of CDX2) correlates to poor prognosis in patients with epithelial ovarian cancer (1). CDH17 may be helpful for early diagnosis of Barrett's esophagus (12). CDH17 has been shown to be a useful marker for distinguishing between primary urinary bladder adenocarcinoma and urothelial carcinoma with glandular differentiation (13). Note that CDH17 does not distinguish primary urinary bladder adenocarcinoma from colorectal adenocarcinoma secondarily involving the bladder (13). In addition, 89% of medullary carcinomas of the colon and rectum were recognized by CDH17 (14). Compared to CDX2 or CK20 alone, the combination of CDX2 and CDH17 is highly sensitive and somewhat specific for colorectal and stomach adenocarcinoma in routine immunohistochemistry, especially in cases with a CK7-/CDX2-/CK20- carcinoma (6,10,14). Data suggests that the combination of CDX2 and CDH17 along with CK7 may improve specificity compared to the panel consisting of CD20, CDX2, villin and CK7 (1-13).

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:
CDX2 (M) + CDH17 (RM) is provided as a prediluted antibody cocktail of anti-CDX2 and anti-CDH17 antibodies, in buffer with carrier protein and preservative.

Antibody	Anti-CDX2	Anti-CDH17
Clone	CDX-88	EP86
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/Antigen	CDX2	CDH17
Cellular Localization	Nuclear	Cytoplasmic/Cell membrane
Staining	Brown (DAB)	Red (Warp Red)

Storage and Stability:
Store at 2ºC to 8ºC. 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; others not tested
Positive Tissue Control: Normal colon or colon cancer

Protocol Recommendations:
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 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 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. Use TBS for washing steps.

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.

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Precautions Cont'd:

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 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.

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. Saad RS, *et al*. CDX2, cytokeratins 7 and 20 immunoreactivity in rectal adenocarcinoma. Appl Immunohistochem Mol Morphol. 2009 May; 17(3):196-201.
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6. Bayrak R, Haltas H, Yenidunya S. The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. Diagn Pathol. 2012 Jan 23; 7:9.
7. Lee MJ, *et al*. CDX-2 expression in malignant germ cell tumors of the testes, intratubular germ cell neoplasia, and normal seminiferous tubules. Tumour Biol. 2012 Dec; 33(6):2185-8.
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9. Huang LP, *et al*. Up-regulation of cadherin 17 and down-regulation of homeodomain protein CDX2 correlate with tumor progression and unfavorable prognosis in epithelial ovarian cancer. Int J Gynecol Cancer. 2012 Sep; 22(7):1170-6.
10. Panarelli NC, *et al*. Tissue-specific cadherin CDH17 is a useful marker of gastrointestinal adenocarcinomas with higher sensitivity than CDX2. Am J Clin Pathol. 2012 Aug; 138(2):211-22.
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12. Mokrowiecka A, *et al*. Liver-intestine-cadherin is a sensitive marker of intestinal differentiation during Barrett's carcinogenesis. Dig Dis Sci. 2013 Mar; 58(3):699-705.

References Cont'd:

13. Rao Q, *et al*. Distinguishing primary adenocarcinoma of the urinary bladder from secondary involvement by colorectal adenocarcinoma: extended immunohistochemical profiles emphasizing novel markers. Mod Pathol. 2013 May; 26(5):725-32.
14. Lin F, *et al*. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med. 2014 Aug; 138 (8):1015-26.
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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