Claudin-4

Concentrated and Prediluted Monoclonal Antibody
Control Number: 901-3121-040819

BIOCARE MEDICAL

Catalog Number:	**ACI 3121 A, B**	**API 3121 AA**
Description:	0.1, 0.5 mL, conc.	6.0 mL RTU
Dilution:	1:100	Ready-to-use
Diluent:	Van Gogh Yellow	N/A

Intended Use:
For In Vitro Diagnostic Use
Claudin-4 [3E2C1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of Claudin-4 protein 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:
Claudin-4 (Clostridium perfringens enterotoxin receptor) is a tight junction protein encoded by the gene CLDN4. Expression of Claudin-4 has been associated with either poor prognosis or a more favorable diagnosis, depending on the type of cancer. Claudin-4 has been shown to distinguish adenocarcinoma from malignant mesothelioma with 99% specificity in malignant effusions (1). Claudin-4 overexpression was able to independently predict survival in a breast cancer multivariate analysis as it was associated with poor prognosis, high tumor grade and Her2 expression and was inversely correlated with estrogen receptor staining (2). In luminal breast cancer, the increase of Claudin-4 protein was correlated with the increase of tumor grade and with Ki-67, and thus demonstrated an overall shorter life survival (3). Basal-like tumors also demonstrated overexpression of Claudin-4 (4). Counter to the above breast cancer subtypes, the presence of Claudin-4 in triple negative breast cancer was a biomarker that demonstrated a favorable prognosis (3). Loss of Claudin-4 was also seen in 69% of advanced gastric cancers and correlated with poor differentiation (5). Low expression of the Claudin-4 protein correlated with higher T staging, lymphatic metastasis and higher risk of recurrence in esophageal squamous cell carcinoma (6). Claudin-4 overexpression in prostate cancer may offer a Claudin-4 targeted therapy as a potential treatment (7).

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 antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: 3E2C1
Isotype: IgG1
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: Synthetic peptide corresponding to a 22 amino acid sequence derived from the C-terminal region of human Claudin-4
Cellular Localization: Cell membrane
Positive Tissue Control: Colon carcinoma or breast carcinoma

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

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

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. 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.
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) (8)
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. (9)
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.

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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:

1. Jo VY, Cibas ES, Pinkus GS. Claudin-4 immunohistochemistry is highly effective in distinguishing adenocarcinoma from malignant mesothelioma in effusion cytology. Cancer Cytopathol. 2014 Apr;122(4):299-306.
2. Lanigan F, *et al*. Increased claudin-4 expression is associated with poor prognosis and high tumour grade in breast cancer. Int J Cancer. 2009 May 1;124(9):2088-97.
3. Kolokytha P, *et al*. Claudin-3 and claudin-4: distinct prognostic significance in triple-negative and luminal breast cancer. Appl Immunohistochem Mol Morphol. 2014;22(2):125-31.
4. Lu S, *et al*. Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype. Mod Pathol. 2013 Apr;26(4):485-95.
5. Lee SK, *et al*. Loss of the tight junction protein claudin 4 correlates with histological growth-pattern and differentiation in advanced gastric adenocarcinoma. Oncol Rep. 2005 Feb;13(2):193-9.
6. Shi M, *et al*. Low expression of claudin-4: an indicator of recurrence in esophageal squamous cell carcinoma after Ivor Lewis esophagectomy? Med Oncol. 2014 May;31(5):951.
7. Maeda T, *et al*. Claudin-4-targeted therapy using Clostridium perfringens enterotoxin for prostate cancer. Prostate. 2012 Mar;72(4):351-60.
8. 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."
9. 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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