

## Anti-Human Claudin-5 [EP224]

Catalog No.	Description
AN718-5M	6 ml of Ready-to-Use Antibody for Use with BioGenex Super Sensitive Detection Systems or Other Equivalent Detection Systems
AN718-10M	10 ml of Ready-to-Use Antibody in a barcode labeled vial for Use with BioGenex Super Sensitive Detection Systems and BioGenex i6000 Automated Staining Systems
NU718-UC	1 ml of Concentrated Antibody for Use with BioGenex Super Sensitive Detection Systems or Other Equivalent Detection Systems
NU718-5UC	0.5 ml of Concentrated Antibody for Use with BioGenex Super Sensitive Detection Systems or Other Equivalent Detection Systems
AY718-YCD	Ready-to-Use Antibody in RFID tagged vial for use on the Xmatrix <sup>®</sup> Elite/Ultra Staining System, 200 tests
AY718-50D	Ready-to-Use Antibody in RFID tagged vial for use on the Xmatrix <sup>®</sup> Elite/Ultra Staining System, 50 tests

Immunogen	Clone	Species	Ig Class
A synthetic peptide corresponding to residues of human Claudin-5 protein	EP224	Rabbit	IgG

### Intended Use

This antibody is currently available for in vitro diagnostic use. This antibody is designed for the specific localization of Claudin 5 in formalin-fixed, paraffin-embedded tissue sections.

### Summary and Explanation

The Claudin superfamily consists of many structurally related proteins in humans. These proteins are important structural and functional components of tight junctions in paracellular transport. Claudins are located in both epithelial and endothelial cells in all tight junction bearing tissues. Claudin-5 is a member of the Claudin family. Mutations in Claudin-5 have been found in patients with velocardiofacial syndrome. Claudin-5 labels endothelial cells. It has been used as a marker for endothelial lesions. Claudin-5 is also found in bronchial and lung epithelial cells. In tumors, Claudin-5 expression has been found in lung adenocarcinoma and squamous carcinoma. In serous ovarian adenocarcinoma increased Claudin-5 expression is associated with aggressive behaviour.

### Principles of the Procedure

The demonstration of antigens by immunohistochemistry (IHC) is a two-step process involving first, the binding of a primary antibody to the antigen of interest, and second, the detection of bound antibody by a chromogen. The primary antibody may be used in IHC using manual techniques or using BioGenex Automated Staining System. BioGenex offers a variety of Super Sensitive detection systems including Link-Label and Polymer-based technologies to detect the chromogenic signal from the stained tissues and cells.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact BioGenex Technical Support at 1.800.421.4149 or your local distributor.

### Reagents Provided

Rabbit Monoclonal Antibody to Claudin-5 is affinity purified and diluted in PBS, pH 7.2, containing 1% BSA and 0.09% sodium azide.

### Dilution of Primary Antibody

BioGenex Ready-to-Use antibodies have been optimized for use with the recommended BioGenex Detection System and should not require further dilution. Further dilution may result in loss of sensitivity and must be validated by the user.

BioGenex Concentrated antibodies must be diluted in accordance with the staining procedure when used with the recommended BioGenex Detection System (see staining procedure section below). Use of any detection methods other than the recommended systems and protocols require validation by the user.

### Materials Required But Not Provided

All the reagents and materials required for IHC are not provided. Pre-treatment reagents, Super Sensitive detection systems, control slides, control reagents and other ancillary reagents are available from BioGenex. Please refer to the product insert(s) of the BioGenex Super Sensitive IHC detection systems for detailed protocols and instructions.

The IHC procedure may need other lab equipment that is not provided including oven or incubator (capable of maintaining 56-60°C), BioGenex [Automated Staining System](#), Humidity Chamber, Microwave oven, Staining Jars or baths, Timer (capable of 3-20 minute intervals), Wash Bottles, Absorbent Wipes, Microscope slides (pre-treated with poly-L-Lysine), Coverslips, Lens paper and Light microscope with magnification of 200X.

### Storage and Handling

Antibodies should be stored at 2-8°C without further dilution. Fresh dilutions, if required, should be made prior to use and are stable for up to one day at room temperature (20-26°C). Unused portions of antibody preparations should be discarded after one day to minimize microbial contamination and increase in nonspecific staining. Diluted antibody preparations can be refrigerated or frozen for extended shelf life.

This antibody is suitable for use until expiry date when stored at 2-8°C. Do not use product after the expiration date printed on vial. If reagents are stored under a condition other than those specified in the package insert, they must be verified by the user (U.S. Congress, 1992).

The presence of precipitate or an unusual odor indicates that the antibody is deteriorating and should not be used.

### Specimen Collection and Preparation

Tissues fixed in 10% (v/v) formalin, prior to paraffin embedding, are suitable for use. For further details on specimen preparation please refer to - Kiernan, 1981: Sheehan & Hrapchak, 1980.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

### Treatment of Tissues Prior to Staining

Pretreatment of tissues, if any, should be done as suggested in the staining procedure section below.

### Precautions

This antibody contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazard Communication Standard and EC Directive 91/155/EC. However, this product contains sodium azide, at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at the product concentrations. However, toxicity information regarding sodium azide at product concentrations has

not been thoroughly investigated. Sodium azide may react with lead or 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 for Occupational Safety and Health, 1976). For more information, a Safety Data Sheet (SDS) for sodium azide in pure form is available upon request. Dispose unused reagents according to local, State and Federal regulations. Wear suitable Personal Protective Equipment, do not pipette reagents by mouth, and avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with copious amounts of water.

Refer to appropriate product inserts for instructions of use and safety information on detection reagents and other materials, which may be used with the antibody.

**Recommended Protocol**

Refer to the following table for conditions specifically recommended for this antibody. Refer to the detection system package insert for guidance on specific staining protocols or other requirements.

Parameter	BioGenex Recommendations
Control Tissue	LUNG SQUAMOUS CA as available with Biogenex FB-718N* & FG-718N*
Recommended Dilution for Concentrated Ab	50 in HK941-YAK
Recommended Pretreatment (manual/i6000)**	EZ-AR2 Elegance (HK547-XAK)
Recommended Pretreatment(Xmatrx)	EZ-AR2 Elegance (HX032-YCD)
Antibody incubation(manual/i6000)	30 mins at RT
Antibody incubation (Xmatrx)	45 mins at 25°C
<u>Detection System</u> for manual, Xmatrx & i6000 -Open systems ***	Use two-Step Super Sensitive™ Polymer-HRP IHC Detection System/DAB available from BioGenex (QD400 for Manual and QD410 for Automation). or For One-Step use Super Sensitive™ Polymer-HRP Detection Kit/DAB, available from BioGenex (QD620 or QD630)

\*FB: positive control barrier slides, FG: positive control non barrier slides. Xmatrx requires barrier slides.

\*\* Pretreatment times will vary based on individual microwave power

\*\*\*For Closed system automation (Xmatrx-Elite, Xmatrx-Ultra & i6000 Elite Dx) – Please refer to the factory protocols provided with the instrument.

**Quality Control**

The recommended positive control tissue for this antibody is Vascular Tissue. The user is advised to use the control tissues available from BioGenex for your Quality Control purpose. Refer to the appropriate detection system package inserts for guidance on general quality control procedures.

**Troubleshooting**

Refer to the troubleshooting section in the package inserts of BioGenex Detection Systems (or other equivalent detection systems) for remedial actions on detection system related issues, or contact BioGenex Technical Support Department at 1-800-421-4149 or your local distributor to report unusual staining.

**Expected Results**

This antibody stains cell junction/membrane in positive cells in formalin-fixed, paraffin-embedded tissue sections. Interpretation of the staining result

is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic product or procedure.

**Limitations of the Procedure**

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983). Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive with horseradish peroxidase systems (Omata et al, 1980). Improper counterstaining and mounting may compromise the interpretation of results.

**Performance Characteristics**

BioGenex has conducted studies to evaluate the performance of the antibody with BioGenex detection systems and accessories. The antibodies have been found to be sensitive and show specific binding to the antigen of interest with minimal to no binding to non-specific tissues or cells. BioGenex antibodies have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

**Bibliography**

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