

## Anti-Annexin A1 [ANXA1/1671]

Catalog No.	Description
AM982-5M	6 ml of Ready-to-Use Antibody for Use with BioGenex Super Sensitive™ Detection Systems or Other Equivalent Detection Systems
AM982-10M	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
MU982-UC	1 ml of Concentrated Antibody for Use with BioGenex Super Sensitive™ Detection Systems or Other Equivalent Detection Systems
MU982-5UC	0.5 ml of Concentrated Antibody for Use with BioGenex Super Sensitive™ Detection Systems or Other Equivalent Detection Systems
AX982-YCD	Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrix® Elite/Ultra Staining System, 200 tests
AX982-50D	Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrix® Elite/Ultra Staining System, 50 tests

Immunogen	Clone	Species	Ig Class
Recombinant full length human Annexin A1 protein	ANXA1/1671	Mouse	IgG2

### Intended Use

For research use only.

This antibody is designed for the specific localization of Annexin A1 in formalin-fixed, paraffin-embedded tissue sections.

Evaluation must be performed by a qualified pathologist.

### Summary and Explanation

Annexin A1, also known as lipocortin I, is a protein that is encoded by the ANXA1 gene in humans. Annexin A1 belongs to the annexin family of Ca<sup>2+</sup>-dependent phospholipid-binding proteins with phospholipase A2 inhibitory activity. In resting conditions, human and mouse immune cells such as neutrophils, monocytes, and macrophages contain high levels of annexin A1 in their cytoplasm. Annexin plays important roles in the innate immune response as an effector of glucocorticoid-mediated responses and regulator of the inflammatory process and has anti-inflammatory activity among its many functions. Annexin A1 also plays a significant role in cancer immunology because it aids in migration and metastasis of tumors. For instance ANXA1's association with NEMO and RIP1 activates NF-κB which in turn promotes breast cancer migration and reduces breast cancer proliferation through a negative feedback. Furthermore, increased expression of annexin A1 is correlated with K-ras mutation in colorectal cancer. Using immunohistochemistry, expression of annexin A1 is seen in the cytoplasm and membrane of Breast Cancer, tonsil, spleen and lymph node tissue cells.

### Principles of the Procedure

Antigen detection by Immunohistochemistry (IHC) is a two-step process. In the first step, the primary antibody binds to the antigen of interest, and second, the antigen antibody binding is detected by a chromogen. The [primary antibody](#) may be used in IHC using manual techniques or 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 always 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

Mouse Monoclonal Antibody Annexin A1 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 materials and reagents necessary for IHC are not provided. Pre-treatment reagents, Super Sensitive Detection Systems, control reagents and control slides, 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 (Aptec coated), Coverslips, Lens paper and Light microscope with magnification of 200X.

### Storage and Handling

Antibodies should be stored at 2-8°C without any further dilution. Fresh dilutions, if required, should be prepared prior to use and are stable and steady for up to one day at room temperature (20-26°C). The antibody should not dry out during its intended incubation period when used as directed. Unused vial of antibody preparations should be discarded after one day to minimize microbial contamination and non-specific 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 (4).

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 [Histological and Histochemical Methods: Theory and Practice](#) (5).

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

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### 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 (6). For more information, a Safety Data Sheet (SDS) for sodium azide in pure form is available upon request. Dispose of 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(7).

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	Spleen tissue as available with Biogenex FB-982M* & FG-982M*
Recommended Dilution for Concentrated Ab	1:20 - 40 in HK941
Recommended Pretreatment (manual/i6000)*	EZ-AR2 (HK522-XAK)
Recommended Pretreatment(Xmatrix)	EZ-AR2 Elegance (HX032-YCD)
Antibody incubation(manual/i6000)	1 Hr at RT
Antibody incubation (Xmatrix)	1 Hr at RT
Detection System for manual, Xmatrix & i6000 -Open systems**	Use two-Step Super Sensitive™ Polymer-HRP IHC Detection System/DAB available from BioGenex (QD400 for Manual and QD410 for Automation).

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

Xmatrix requires barrier slides.

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

\*\*\*For Closed system automation (Xmatrix-Elite, Xmatrix-Ultra & i6000 Diagnostics)

– Please refer to the factory protocols provided with the instrument.

### Quality Control

The suggested positive control for this particular antibody is spleen tissue. The user is always advised to use the control tissues. 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 cytoplasmic 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 procedure relating both histological and immunological detection. Improper Tissue handling and processing prior to immunostaining can lead to inconsistent results. Variations in embedding and fixation or the nature of the tissue may lead to variations in results. Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and tissue biotin may result in non-specific staining based on the detection system employed. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive with horseradish peroxidase systems(8). Improper counterstaining and mounting may compromise the interpretation of results.

### Performance Characteristics

BioGenex has conducted studies to evaluate and determine the performance of the antibody with BioGenex detection systems and accessories. The antibodies have been found to be very specific and sensitive and they 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

- Gavins FN and Hickey MJ. “Annexin A1 and the regulation of innate and adaptive immunity”. Front Immunol. 2012 Nov 27;3:354.
- Bist, P et al. “Annexin-1 interacts with NEMO and RIP1 to constitutively activate IKK complex and NF- $\kappa$ B: implication in breast cancer metastasis”. Oncogene. 2011 Jul 14;30(28):3174-85.
- Kang H, Ko J, Jang SW. “The role of annexin A1 in expression of matrix metalloproteinase-9 and invasion of breast cancer cells”. Biochem Biophys Res Commun. 2012 Jun 22;423(1):188-94.
- Yang, YH et al. “Deficiency of annexin A1 in CD4+ T cells exacerbates T cell-dependent inflammation”. J Immunol. 2013 Feb 1;190(3):997-1007.
- Tu, Y et al. “Annexin A1 influences in breast cancer: Controversies on contributions to tumour, host and immunoeediting processes”. Pharmacol Res. 2017 May;119:278-288.

	Temperature Limitation		Manufacturer
	Use By Date		Batch Code
	Non-Sterile		Consult Instructions for Use

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