

Anti-Hemoglobin A

| Catalog No. | Description |
|-------------|---|
| AN977-5M | 6 ml of Ready-to-Use Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system |
| AN977-10M | 10 ml of Ready-to-Use Antibody in a barcode labeled vial for use with BioGenex Super Sensitive™ Detection Systems and i6000™ Automated Staining Systems |
| NU977-UC | 1 ml of Concentrated Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system |
| NU977-5UC | 0.5 ml of Concentrated Antibody for use with BioGenex Super Sensitive™ Detection Systems OR equivalent detection system |
| AY977-YCD | Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx® Elite Staining System, 160 tests |
| AY977-50D | Ready-to-Use Antibody in Barcode labeled vial for use on the Xmatrx® Elite Staining System, 50 tests |
| AX977-4M | Ready-to-Use Antibody in Barcode labeled vial for use on the NanoVIP® Staining System, 50 tests |

| Clone | Species | Ig Class |
|---------|---------|----------|
| EPR3608 | Rabbit | IgG |

Intended Use

For In Vitro Diagnostic Use. This antibody is designed for the specific localization of human Hemoglobin in formalin-fixed, paraffin-embedded (FFPE) tissue sections. Evaluation must be performed by a qualified pathologist.

Summary and Explanation

The human alpha globin gene cluster located on chromosome 16 spans about 30 kb and includes seven loci: 5'- zeta - pseudozeta - mu - pseudoalpha-1 - alpha-2 - alpha-1 - theta - 3'. The alpha-2 (HBA2) and alpha-1 (HBA1) coding sequences are identical and only differ slightly over the 5' untranslated regions and the introns (although they differ significantly over the 3' untranslated regions). Two alpha chains plus two beta chains constitute HbA, which in normal adult life comprises about 97% of the total hemoglobin; alpha chains combine with delta chains to constitute HbA-2, which with HbF (fetal hemoglobin) makes up the remaining 3% of adult hemoglobin. A reduction in the production of normal alpha globin chains results in inherited hematologic disorders such as alpha thalassemia whereby there is an increase in beta chains to compensate for the lack of normal alpha globin chains.

Storage and Handling

Store at 2-8°C. 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). Diluted antibody preparations can be refrigerated or frozen for extended shelf life.

Principles of the Procedure

Antigen detection by immunohistochemistry (IHC) is a two-step process wherein the primary antibody binds to the antigen of interest and that binding is detected by a chromogen. The [primary antibody](#) may be used in IHC using manual techniques or BioGenex Automated Staining System. Positive and negative controls should always be run simultaneously with all patient specimens.

Reagents Provided

Rabbit Monoclonal Antibody to human Hemoglobin Ais 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.

BioGenex concentrated antibodies must be diluted in accordance with the recommended protocol when used with the recommended BioGenex Detection System.

Recommended Protocol

Refer to the following table for conditions specifically recommended for this antibody. Refer to the BioGenex website for guidance on specific staining protocols or other requirements.

| Parameter | BioGenex Recommendations |
|--|---|
| Control Tissue | Spleen tissue as available with Biogenex FB-977NE* & FG-977NE* |
| Recommended Dilution for Concentrated Antibody | 1:50 in HK941 |
| Recommended Pretreatment (Manual/i6000)** | EZ-AR2 (HK522-XAK) |
| Recommended Pretreatment (Xmatrx & Nano VIP) | EZ-AR2 Elegance (HX032-YCD & HX046-08XN) |
| Antibody Incubation (Manual/i6000) | 30-60 Min at RT |
| Antibody Incubation (Xmatrx & Nano VIP) | 45-60 Min at 25°C |
| Detection System for Manual, Xmatrx, Nano VIP & i6000 systems*** | Use BioGenex Two-Step OR One-Step Super Sensitive™ Polymer-HRP IHC Detection System/DAB; see p. 2 for more information |

| | | | |
|--------------|-------------|--------------|-------------|
| Category | Antibodies | Revision No. | G |
| Document No. | 932-977N-EN | Release Date | 17-Jun-2024 |

*FB: positive control micro chamber slides, FG: positive control microscopic slides. Xmatrix & Nano VIP requires micro chamber slides.

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

***For automation systems (Xmatrix-Elite, Nano VIP & i6000 Diagnostics), refer to the factory protocols provided with the instrument.

| Detection System | Two-Step HRP Kit | One-Step HRP Kit | Link and Label Kit |
|--|-------------------------|-------------------------|------------------------|
| Manual | QD440-XAKEN (1000 Test) | QD630-XAKEN (1000 Test) | QP300-XAKE (1000 Test) |
| | QD430-XAKEN (1000 Test) | | |
| | QD420-YIKEN (500 Test) | QD620-XAKEN (500 Test) | QP900-9LE (500 Test) |
| | QD400-60KEN (60 Test) | | |
| Xmatrix - Automation | QD550-YCDEN (200 Test) | QD610-YADEN (200 Test) | N/A |
| NanoVIP- Automation | QD551-YCDEN (100 Test) | QD611-YADEN (100 Test) | N/A |
| i6000 - Automation | QD410-YAXEN (200 Test) | QD610-YAXEN (200 Test) | N/A |
| For more information, visit www.biogenex.com . | | | |

Precautions

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, but proper handling protocols should be observed. For more information, a Safety Data Sheet (SDS) for sodium azide 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.

Quality Control

Refer to BioGenex detection system documents for guidance on general quality control procedures.

Troubleshooting

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

Expected Results

This antibody stains membrane /cytoplasm in positive cells in formalin-fixed, paraffin embedded tissue sections. An example image of a tissue section stained with this antibody can be found

on the product page on the BioGenex website. Interpretation of the staining result is solely the responsibility of the user. Experimental results should be confirmed by a medically-established diagnostic product or procedure.

Limitations of the Procedure

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 pseudo peroxidase 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. Improper counterstaining and mounting may compromise the interpretation of results.

Bibliography

- Woon-Shick, A et al. Identification of hemoglobin-alpha and -beta subunits as potential serum biomarkers for the diagnosis and prognosis of ovarian cancer. *Cancer Sci.* 2005 Mar; 96(3):197-201.
- Abdullah, et al. The Free Alpha-Hemoglobin: A Promising Biomarker for β -Thalassemia. *J MolBiomarkDiagn* 2014, 5:5.
- Petrakos, G. Pregnancy in women with thalassemia: challenges and solutions. *Int J Womens Health.* 2016 Sep 8; 8:441-51.
- Nathan, GB & Gunn, RB. Thalassemia: the consequences of unbalanced hemoglobin synthesis. *Am J Med.* 1966 Nov; 41(5):815-3.

| | | | |
|---|--|---|------------------------------------|
|  | Temperature Limitation | IVD | In Vitro Diagnostic Medical Device |
|  | Use By Date | LOT | Batch Code |
|  | Non-Sterile |  | Consult Instructions for Use |
| EC REP | Representative in the European Community |  | Manufacturer |

© 2020, BioGenex Laboratories. All rights reserved.

| | | | |
|---------------------|-------------|---------------------|-------------|
| Category | Antibodies | Revision No. | G |
| Document No. | 932-977N-EN | Release Date | 17-Jun-2024 |