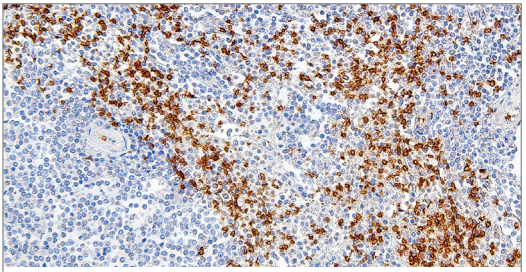
GeneAb™ Monoclonal Mouse Anti-Human

Glycophorin A Antibody

Clone: IHC587
Positive Control: Spleen, Bone Marrow tissues

Source: Mouse Monoclonal
Localization: Membranous



GeneAb™ Glycophorin A [IHC587] on Spleen tissue

Product Information

REF	Description
IHC587-100	0.1 ml, Concentrate
IHC587-1	1 ml, Concentrate
IHC587-7	7 ml, Predilute
IHC587-25	25 ml, Predilute
IHC587-cS	20 µl, Concentrate Sample
IHC587-pS	1 ml, Predilute Sample
IHC587-PC	3 Positive Control Slides

1. Intended Use

This antibody is intended for *in vitro* diagnostic (IVD) use.

The Glycophorin A [IHC587] antibody is intended for qualified laboratories to qualitatively identify by light microscopy, the presence of Glycophorin A expression in formalin-fixed, paraffin-embedded (FFPE) tissue sections using immunohistochemistry assay. Use of this antibody is indicated when there is a need to know the existence of the protein specific cell type and expression levels requested by a qualified pathologist.

2. Summary and Explanation

Glycophorin A (GPA) and Glycophorin B (GPB) are erythrocyte blood group determinants that minimize erythrocyte aggregation during the circulation of blood. Detection of Glycophorin A by immunohistochemistry provides the information of Glycophorin A expression level in testing tissue samples.

3. Principles and Procedures

Visualization of the antigen present in tissue sections is accomplished in a multi-step immunohistochemical staining process, in conjunction with a horseradish peroxidase (HRP) or alkaline phosphatase (AP) linked detection system. The process involves the addition of the stated antibody (primary antibody) to a tissue slide, followed by a secondary antibody (linked to an enzyme complex) which specifically binds to the primary antibody. A chromogenic substrate is then added which reacts with the enzyme complex, resulting in a colorimetric reaction at the site of the antigen. Results are interpreted using a light microscope.

4. Materials and Methods

Product Format	Dilution	Buffer Composition
Predilute	Ready to Use	GenomeMe Antibody Diluent (Cat# IHC000)
Concentrate	1:50-1:200	Tris Buffer, pH 7.3 - 7.7, with 1% BSA and <0.1% Sodium Azide

Reconstitution, Mixing, Dilution, and Titration

The prediluted antibody does not require any mixing, dilution, reconstitution, or titration; the antibody is ready-to-use and optimized for staining. Any further dilution may affect the quality of the staining signal or antibody-antigen interaction.

The concentrated antibody requires dilution using an Antibody Diluent Buffer, to the recommended working dilution range listed in the table above, prior to use.

Storage and Handling

Store at 2-8°C. To ensure stability, immediately replace vial back in the refrigerator after each use. When stored correctly, the antibody is stable until the expiry date indicated on the label. Positive and negative controls should be concurrently

run with tissue specimens, to enable identification of any inadequacies with the antibody or reagents. If antibody stability issues are suspected, please contact GenomeMe Customer Service at **info@genomeme.ca**.

Specimen Collection and Preparation for Analysis

Each tissue section should be fixed with 10% neutral buffered formalin, cut to the applicable thickness (4µm), and placed on a glass slide that is positively charged. The prepared slide should then be baked for a minimum of 30 minutes in a 53-65°C oven (do not exceed 24 hours).

Note: Performance evaluation has been shown on human tissues only. Variable results may occur due to extended fixation time or variations in tissue preparation. Do not use alcohol containing fixatives as those may result in a loss of staining activity.

Material Required but not Provided

The following materials are required but are not provided:

a) Detection system (ie. BOND Polymer Refine Detection Kit or UltraView/OptiView Universal DAB Detection Kit)
b) Chromogen (ie. DAB Substrate Kit)
c) IHC wash buffer and blocking solution
d) Hematoxylin or other counterstaining reagents
e) Ethanol or reagent alcohol, xylene or xylene substitute and mounting medium
f) Antibody diluents
g) Positive and negative control tissue

5. Instructions For Use

Automated Staining with Leica Biosystems Bond-MAX Platform:

This primary antibody has been optimized and validated using the Leica Bond-MAX Fully Automated IHC & ISH Stainer, applying IHC Protocol F. Heat-induced epitope retrieval (HIER) is recommended using ER2 for 20-30 minutes. Antibody concentrate dilution range is 1:50-1:200.

Automated Staining with Ventana BenchMark ULTRA Platform:

This primary antibody has been optimized and validated using the Ventana BenchMark ULTRA IHC/ISH System. Antibody concentrate dilution range is 1:100-1:200.

Recommended protocol parameters are as follows:

a) Detection Kit: UltraView DAB IHC
b) Pretreatment Protocol: CC1 64 minutes, 100°C
c) Primary Antibody: 32 minutes, 36-37°C

For all other automated IHC staining systems, refer to the corresponding user manual for specific instructions.

Manual Use:

The following is a recommended starting protocol, users must optimize based on their specific detection kit and method.

1. **Pretreatment:** Perform heat-induced epitope retrieval (HIER) at pH 9, with a pressure cooker for 1.5-3 minutes.
2. **Blocking:** For HRP, block with peroxidase blocking solution for 10-15 minutes at room temperature. If a different system than HRP is used, an alternative blocking solution will be required.
3. **Primary Antibody:** Apply the antibody at a dilution of 1:50-1:100 and incubate for 30-60 minutes at room temperature or overnight at 4°C.
4. **Secondary Antibody:** Apply an appropriate HRP conjugated secondary antibody for 20-30 minutes at room temperature. If different system than HRP is used, an alternatively conjugated secondary antibody will be required.
5. **Substrate Development:** Apply and incubate with DAB for 5-10 minutes at room temperature.
6. **Counterstaining:** Counterstain with hematoxylin for 0.5-5 minutes, based on the instructions for the hematoxylin used. Rinse with distilled water and bluing solution for 30 seconds.
7. Dehydrate and apply coverslip.

6. Quality Control Procedures and Interpretation of Results

The immunohistochemical staining process results in a colorimetric reaction at the site of the antigen, localized by the primary antibody. A qualified pathologist must interpret the tissue specimen results only after the positive and negative control tissues have been analyzed. It is recommended to include a set of tissue controls with each staining run to monitor for antibody, tissue, and reagent performance. Tissue sections may contain both positive and negative staining elements. In these cases and where applicable, these sections may serve as both the positive and negative tissue control.

Positive Control Tissue

A positive control tissue should be processed in the same manner as the specimen and run with each test condition to provide control for variables such as tissue processing, fixation, and staining. It should function to provide validity to the specimen results obtained and can consist of fresh autopsy, biopsy, or surgical tissue. Once stained, the positive control tissue should analyzed first to ensure that the antibody and all reagents are performing as intended. Counterstaining will result in a blue coloration, which may range from pale to dark depending on the length of the incubation time and potency of the hematoxylin. If positive staining is not observed, the positive control tissue must be deemed invalid and the results obtained with the tissue specimen must also be treated as such.

Positive tissue control: Spleen and bone marrow tissues

Negative Control Tissue

Some tissue sections can also function as an internal negative control due to the diversity of staining elements present. This, however, should first be confirmed by the user.

Negative tissue control: Colon, breast and brain tissues

Tissue Specimens

Tissue specimens should only be analyzed after the positive and negative control tissues have been deemed valid. Negative staining indicates that the antigen was not detected in the tissue while positive staining represents the presence of the antigen. A tissue section stained with hematoxylin and eosin should be used to analyze the morphology of the tissue specimen and verified by a qualified pathologist.

Peformance Characteristics

This antibody has been validated by immunohistochemistry using a FFPE human tissue microarray comprised of different types of tissues. Positive staining was observed in spleen and bone marrow tissues. No staining was seen in colon, breast and brain tissues. A representative positive staining image is shown on Page 1.

Analytical Performance

Trueness of Measure, Precision of Measure, repeatability and reproducibility of the antibody, Analytical Specificity were analyzed using tissue samples from peer-reviewed, published literature known to be either positive or negative, and this study found no unexpected results.

The positive tissue control is spleen and bone marrow tissues, and negative tissue control is colon, breast and brain tissues.

Clinical Performance

The data from the Analytical Performance studies used samples obtained from authorized donors and patients, and demonstrates the antibody product performance matches that of established, routine diagnostic testing materials and scientific peer-reviewed literature, validating its clinical setting performance.

7. Troubleshooting

1. If tissue sections wash off the slide, this may be caused by:
    a) Slides are not positively charged.
    b) Inadequate neutral-buffering of the formalin used for the fixation process.
    c) A thick tissue section.
    d) Inadequate drying of the tissue section prior to staining.

2. If the positive control tissue exhibits negative staining, this may be due to:
    a) An issue with the primary antibody or one of the secondary reagents.
    b) Improper collection, fixation or deparaffinization of the tissue section.
    c) Errors in the IHC staining process.

3. If the positive control tissue exhibits weaker staining than expected, this may be due to sub-optimal IHC conditions, partial degradation of the primary antibody or improper storage of secondary reagents. Analysis of the positive and/or negative control tissues can help with determining the cause.

For assistance with all other types of inquiries, please contact GenomeMe Customer Service at **info@ genomeme.ca.**

8. Limitations

1. This antibody is intended for *in vitro* diagnostic (IVD) use by qualified personnel in laboratories only.
2. Due to biological variability inherent to the expression of certain antigens and immunohistochemical procedures, appropriate positive and negative controls should be used alongside the tissue specimen. Staining and interpretation of results should be conducted in a certified, licensed laboratory, under the supervision and responsibility of a qualified pathologist.
3. This antibody, when used with the appropriate detection systems and reagents, detects antigen(s) that remain intact through the tissue fixation, processing and sectioning as described. Any deviation from these recommended procedures or improper handling may compromise the validity and/or analysis of the results. Do not use alcohol containing fixatives as those may result in a loss of staining activity.
4. GenomeMe provides prediluted antibodies in a ready-to-use, optimally diluted format for use as instructed. Due to the potential for variation in tissue processing and fixation, it may be necessary to adjust the incubation time of the primary antibody for different tissue specimens.
5. GenomeMe provides concentrated antibodies in a format that requires dilution with GenomeMe Antibody Diluent. Use of a diluent different than that specified in the package insert must be validated by the user to ensure proper compatibility with the antibody.
6. The tissue specimen staining results must also take into account any clinical correlation with the patient's medical history and other diagnostic information. The user is responsible for the interpretation of results within the context of the patient.
7. Any discrepancies or unexplained results in control or tissue specimens can be reported to GenomeMe Customer Service at info@genomeme.ca for further assistance. Please refer to the troubleshooting section for common causes of issues.
8. False positive results may occur in tissue specimens due to the possibility of non-immmunological binding of substrate reaction products or proteins. False positive results may also occur subject to the type of immunostaining technique used, or due to the activity of pseudoperoxidase, endogenous peroxidase, or endogenous biotin.
9. Due to the effect of autoantibodies or natural antibodies, normal sera from an animal source that is the same as the secondary antisera may result in false negative or false positive results when used in blocking steps.
10. Non-specific staining with horseradish peroxidase may be observed when using tissues containing hepatitis B surface antigen due to the patient's infection with the hepatitis B virus.

9. Warnings and Precautions

1. Ensure proper reagent handling procedures are followed. Always wear laboratory coats, use disposable gloves and other appropriate personal protective equipment when handling reagents.
2. Do not ingest any antibody or reagent. Avoid contact with eyes and other mucous membranes. Should any contact occur, rinse the area with copious amounts of water and follow laboratory procedures for reporting the exposure.
3. All incubation times and temperatures must be validated by the user with first use. Any usage or storage conditions different than that specified on the package insert should also be validated by the user.
4. Treat all tissue specimens, patient autopsy/biopsy/surgical samples and any materials in contact with these as potentially biohazardous materials and handle with appropriate laboratory precautions.
5. To ensure antibody stability and the accuracy of results, ensure microbial contamination of the antibody does not occur.
6. Monitor for any changes in appearance, or clouding, of the antibody product, as this may be a sign of degradation or other contamination which will affect its efficacy.

10. References

1. Rollins-Raval MA, et al. Am J Clin Pathol. 2012; 137:30-8. 2. Dong HY, et al. Am J Surg Pathol. 2011; 35:723-32. 3. Sadahira Y, et al. J Clin Pathol. 1999; 52:919-21. 4. Chang CC, et al. Am J Clin Pathol. 2000; 114:807-11.

11. Symbols

GenomeMe uses the following symbols and signs in addition to those listed in the ISO15223-1 standard: VOL Volume of Product

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