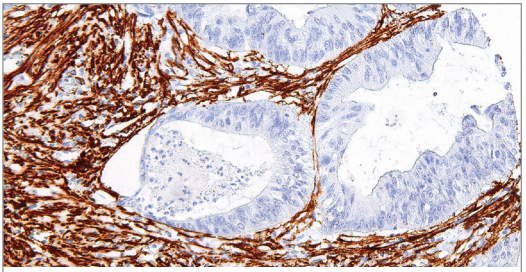
GeneAb™ Monoclonal Mouse Anti-Human

Actin, Smooth Muscle Antibody

Clone: IHC506
Positive Control: Appendix, Uterus, Vessel Wall, Colon

Source: Mouse Monoclonal
Localization: Cytoplasmic



GeneAb™ Actin, Smooth Muscle [IHC506] on Colon

Product Information	
REF	**Description**
IHC506-100	0.1 ml, Concentrate
IHC506-1	1 ml, Concentrate
IHC506-7	7 ml, Predilute
IHC506-25	25 ml, Predilute
IHC506-cS	20 µl, Concentrate Sample
IHC506-pS	1 ml, Predilute Sample
IHC506-PC	3 Positive Control Slides

1. Intended Use

This antibody is intended for Research Use Only.

The Actin, Smooth Muscle [IHC506] antibody is intended for qualified laboratories to qualitatively identify by light microscopy, the presence of associated antigens in formalin-fixed, paraffin-embedded (FFPE) tissue sections using immunohistochemistry test methods. Use of this antibody is indicated as an aid in the identification Actin, Smooth Muscle strictly for research purposes.

2. Summary and Explanation

Actin is part of the cytoskeletal system of all cell types. Smooth muscle actin is found in myofibroblasts and myoepithelium, but not in cardiac or skeletal muscles. Labeling of smooth muscle actin in concert with muscle specific actin staining can allow for differentiation between rhabdomyosarcoma and leiomyosarcoma, as muscle-specific actin is found in rhabdomyoblasts, while smooth muscle actin is found in leiomyosarcomas.

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 not recommended. Antibody concentrate dilution range is 1:100-1:200.

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 6, 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. The tissue specimen result should be interpreted 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.

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. Tissue components that do not stain should demonstrate an absence of specific staining. If specific staining is observed, the negative control tissue must be deemed invalid and the results obtained with the tissue specimen must also be treated as such.

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 FFPE human tissue microarray sections comprised of different types of normal and cancerous tissues. Strong Positive staining was observed in all smooth muscle cells in vessels. No staining was observed on epithelial cells, hepatocytes, lymphocytes, colorectal and breast cancer cells. A representative positive staining image is shown on Page 1.

Analytical Performance

Trueness of Measure was analyzed using tissue samples from peer-reviewed, published literature known to be either positive or negative, and this study found no unexpected results. Precision of Measure analysis involved known positive tissue samples performed to assess the repeatability and reproducibility of the antibody product over time and in different lots; standard acceptance criteria were met in all studies. Analytical Specificity testing found the only known interference comes from alcohol containing fixatives, which demonstrated a loss of staining activity and thus should not be used in conjunction with the processing of samples to be stained using this product. Analytical Sensitivity was tested using randomly chosen tissues representing different levels of antigen expression, a range of staining intensities was observed illustrating the product is sensitive to staining a diverse range of expression levels. Limits of Detection, Measurement Range and Linearity of Measure are all unable to be defined for qualitative and non-linear products such as this. For more in depth data from the above mentioned studies please see the Technical Documentation for this product.

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 Research Use Only 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.
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. 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.
7. 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.
8. 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.
9. 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. Cooke PH. J Cell Biol. 1976; 68:539-56. 2. Gown AM, et al. J Cell Biol. 1985; 100:807-13. 3. Lazarides E. J Histochem Cytochem. 1975; 223:507-28.