GATA-3
Concentrated and Prediluted Monoclonal Antibody
901-405-011817

<table>
<thead>
<tr>
<th>Catalog Number:</th>
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<tr>
<td>CM 405 A, B</td>
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<td>PM 405 AA</td>
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<td>OAI 405 T60</td>
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| Description:   | 0.1, 0.5 ml, concentrated | 6.0 ml, predilute | 60 tests, predilute |
| Dilution:      | 1:100                      | Ready-to-use      | Ready-to-use        |
| Diluent:       | Van Gogh Yellow            | N/A               | N/A                 |

Intended Use:
For In Vitro Diagnostic Use
GATA-3 [L50-823] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of GATA binding protein 3 by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
GATA-3 (GATA binding protein 3) is a member of the GATA family of transcription factors. This 50 kDa nuclear protein regulates the development and subsequent maintenance of multiple tissues. Studies have shown GATA-3 orchestrates gene expression profiles during embryogenesis of a variety of human tissues, including hematopoietic cells, skin, kidney, mammary gland, and the central nervous system (1-5). Among several other roles, GATA-3 has been identified as a key player of luminal cell differentiation in the mammary gland (4). GATA-3 appears to control a set of genes involved in the differentiation and proliferation of breast cancer (3-4). The expression of GATA-3 has a strong association with the expression of estrogen receptor-alpha (ER) in breast cancer, and there is mounting evidence that GATA-3 can be used as a clinical marker to determine response to hormonal therapy and to refine the prognosis of breast cancer patients (3,4,6). GATA-3 has also been shown to be a novel marker for bladder cancer (1-2). In one study, GATA-3 stained 67% of 308 urethelial carcinomas, but none for prostate or renal carcinomas (1).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human
Clone: L50-823
Isotype: IgG1/kappa

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Peptide between trans-activation and DNA-binding domains of GATA-3

Cellular Localization: Nuclear

Positive Control: Bladder cancer and breast cancer

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (manual use):
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment Solution (recommended): Reveal

Pretreatment Protocol:
Heat Retrieval Method:
For bladder cancer, retrieve sections under pressure using Biocare's NxGen Decloaking Chamber at 110°C for 15 minutes; alternatively, follow the recommendations in the Decloaking Chamber User Manual if using a different model. For breast cancer, Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated solution for 5 minutes. In the case of using Biocare's Decloaking Chamber Pro or Decloaking Chamber Plus. If using Biocare's Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

Protein Block (Optional): Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Protocol Recommendations (ONCORE Automated Slide Staining System):
OAI405 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

Protocol Name: GATA-3
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR2, low pH; 101°C
Reagent Name, Time, Temp.: GATA-3, 30 min., 25°C

Technical Note:
This antibody has been optimized for use with Biocare's MACH 4 Universal HRP-Polymer Detection and ONCORE HRP Detection. Use TBS for washing steps.

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to: fixed, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to
Limitations Cont’d:

determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and 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 of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare’s Technical Support at 1-800-542-2002.

References:

References Cont’d: