

GATA-3

Concentrated and Prediluted Monoclonal Antibody
901-405-060223

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Available Product Formats

Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 405 A, B	0.1, 0.5 mL	1:100	Van Gogh Yellow
Predilute	PM 405 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 405 T60	60 tests	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALI 405 G7	7.0 mL	Ready-to-use	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 urothelial 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. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human

Clone: L50-823

Isotype: IgG1/kappa

Protein Concentration: Call for lot specific Ig concentration.

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

Cellular Localization: Nuclear

Positive Tissue 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration

date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM405 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Deparaffinization: Deparaffinize for 8 minutes with Val DePar.

Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).

Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.

Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.

Primary Antibody: Incubate for 30 minutes.

Secondary: Incubate for 10 minutes with Val Mouse Secondary.

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 10 minutes with Val Universal Polymer.

Chromogen: Incubate for 5 minutes with Val DAB.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment Solution (recommended): Reveal

Pretreatment Protocol:

Heat Retrieval Method:

For bladder cancer, retrieve sections under pressure using 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 if using Decloaking Chamber Pro or Decloaking Chamber Plus. If using 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 5-10 minutes at RT with 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 Warp Red.

Counterstain:

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

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI405 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: GATA-3

Protocol Template (Description): Ms HRP Template 1

Dewaxing (DS Option): DS2

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Antigen Retrieval (AR Option): AR2, low pH; 101°C
Reagent Name, Time, Temp.: GATA-3, 30 min., 25°C

Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI405 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: GATA-3
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR2, low pH; 95°C
Block Option: Buffer
Reagent Name, Time, Temp.: GATA-3, 15 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):

PM405 is compatible for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):

ALI405 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Protocol Name: IHC Protocol F
Detection: Bond Polymer Refine
HIER: 20 min with ER1
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, 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 determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

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:

1. Raspollini MR, *et al.* The use of placental S100 (S100P), GATA3 and Napsin A in the differential diagnosis of primary adenocarcinoma of the bladder and bladder metastasis from adenocarcinoma of the lung. *Pathologica*. 2010 Feb; 102(1):33-5.
2. Esheba GE, *et al.* Expression of the urothelial differentiation markers GATA3 and placental S100 (S100P) in female genital tract transitional cell proliferations. *Am J Surg Pathol*. 2009 Mar; 33(3):347-53.
3. Albergaria A, *et al.* Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours. *Breast Cancer Res*. 2009; 11(3):R40.
4. Kouros-Mehr H, *et al.* GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. *Cancer Cell*. 2008 Feb; 13(2):141-52.
5. Voduc D, *et al.* GATA-3 expression in breast cancer has a strong association with estrogen receptor but lacks independent prognostic value. *Cancer Epidemiol Biomarkers Prev*. 2008 Feb; 17(2):365-73.
6. Parikh P, *et al.* GATA-3 expression as a predictor of hormone response in breast cancer. *J Am Coll Surg*. 2005 May; 200(5):705-10.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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