BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Purified Mouse anti-GATA3

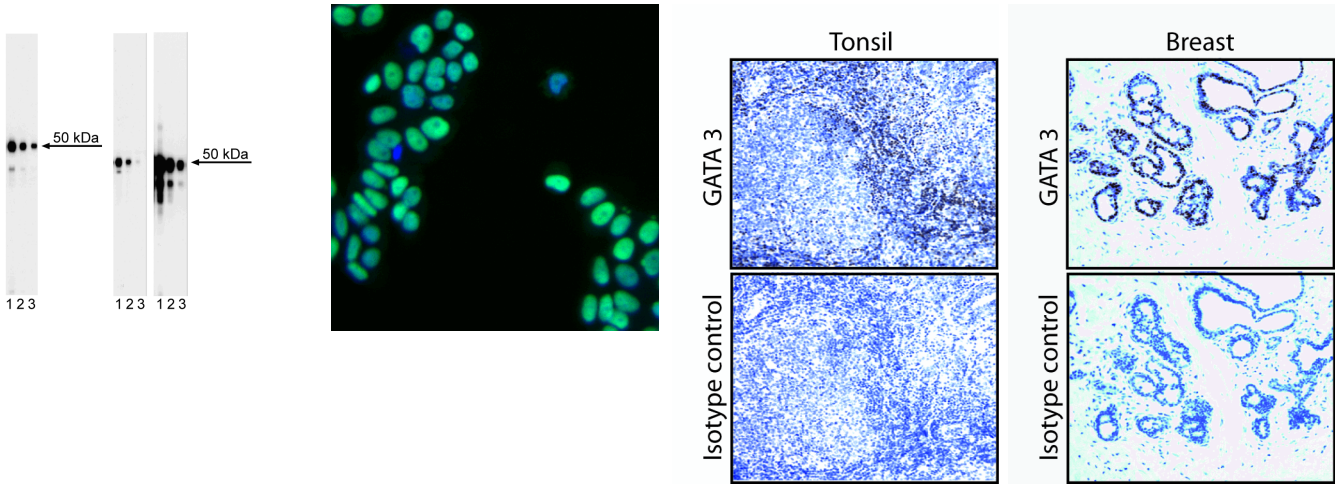
Product Information

Material Number:	**558686**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	L50-823
Immunogen:	Conserved peptide between the trans-activation and DNA-binding domains of human, mouse and rat GATA3
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed: Human, Mouse Predicted: Rat
Target MW:	50 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

GATA3 (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. GATA3 is involved in the development of T lymphocytes (regulates T cell receptor subunit gene expression) and the differentiation of mature T cells to become Th2 cells. The expressed levels of normal or mutant GATA3 are also associated with the behaviors of various cancer cells including estrogen receptor-positive breast carcinoma cells.

The L50-823 monoclonal antibody recognizes human and mouse GATA3.



FAR LEFT, ***Western blot analysis of GATA3 in human T leukemia and mouse T helper cells.*** *Left panel: Jurkat cell lysate (Cat. No. 611451) was probed with Mouse anti-GATA3 monoclonal antibody at concentrations of 0.0156 (lane 1), 0.0078 (lane 2), and 0.0039 µg/ml (lane 3). Middle panel: 2D6 (mouse Th1) cell lysate was probed with Mouse anti-GATA3 monoclonal antibody at concentrations of 0.2500 (lane 1), 0.0625 (lane 2), and 0.0156 µg/ml (lane 3). Right panel: D10.G4.1 (mouse Th2, ATCC TIB -224) cell lysate was probed with Mouse anti-GATA3 monoclonal antibody at concentrations of 0.0625 (lane 1), 0.0156 (lane 2), and 0.0039 µg/ml (lane 3). GATA3 is identified as a band of 50 kDa.*

MIDDLE LEFT, ***Immunofluorescent staining of human breast adenocarcinoma.*** *MCF-7 cells (ATCC HTB-22) were cultured, fixed, permeabilized with cold methanol, stained with Purified Mouse anti-Human GATA3 monoclonal antibody (pseudo-colored green), and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 647 goat anti-mouse Ig (Invitrogen). The images were captured on a BD Pathway™ 435 Bioimager System with a 20x objective and merged using BD Attovision™ software.*

RIGHT, ***GATA3 staining on human tonsil and breast.*** *Following antigen retrieval with BD Retrievagen A buffer (Cat. no. 550524), the formalin-fixed paraffin-embedded sections were stained with either Purified Mouse anti-GATA3 monoclonal antibody (top panel) or Purified Mouse IgG1 κ monoclonal isotype control (bottom panel, Cat. No. 550878), with Hematoxylin counterstaining. GATA3 is detected in the nuclei of the T lymphocytes between the lymphoid follicles of the tonsil and in the nuclei of the cuboidal epithelium of the mammary secretory tubules. Original magnification: 20X.*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

BD Biosciences

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development

Recommended Assay Procedure:

Methanol Procedure for a 96-well plate, with nuclear counterstain:

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells by adding 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubating for 5 minutes at RT.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (3% FBS in 1× PBS) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well and incubating for 30 minutes at RT.
6. Remove the blocking buffer, dilute the antibody in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, dilute the second-step reagent in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted second-step reagent to each well and incubating for 1 hour at RT.
9. Remove the diluted second-step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
550524	Retrievagen A (pH 6.0)	1000 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.

References

Kouros-Mehr H, Slorach EM, Sternlicht MD, Werb Z. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell.* 2006; 127:1041-1055. (Biology)

Marine J, Winoto A. The human enhancer-binding protein Gata3 binds to several T-cell receptor regulatory elements. *Proc Natl Acad Sci U S A.* 1991; 88(16):7284-7288. (Biology)

Steenbergen RDM, OudeEngberink VE, Kramer D, et al. Down-regulation of GATA-3 expression during human papillomavirus-mediated immortalization and cervical carcinogenesis. *Am J Pathol.* 2002; 160(6):1945-1951. (Biology)

Usary J, Llaca V, Karaca G, et al. Mutation of GATA3 in human breast tumors. *Oncogene.* 2004; 23(46):7669-7678. (Biology)

van Esch H, Groenen P, Nesbit MA, et al. GATA3 haplo-insufficiency causes human HDR syndrome. *Nature.* 2000; 106:419-422. (Biology)

Yang Z, Gu L, Romeo P-H, et al. Human GATA-3 trans-activation, DNA-binding, and nuclear localization activities are organized into distinct structural domains. *Mol Cell Biol.* 1994; 14(3):2201-2212. (Biology)

Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell.* 1997; 89(4):587-596. (Biology)