BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Alexa Fluor® 488 Mouse anti-GATA3

Product Information

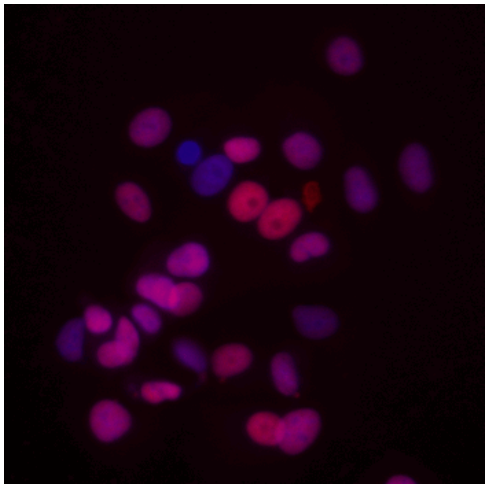
Material Number:	**560077**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	L50-823
Immunogen:	Conserved peptide between the trans-activation and DNA-binding domains of human, mouse and rat GATA3
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed by Bioimaging: Human Confirmed by western blot using purified antibody (Cat. No. 558686): Human, Mouse Predicted: Rat
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤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.
This antibody conjugate is routinely tested and optimized for Bioimaging. For flow cytometry, we recommend these alternate products:

Fluorochrome conjugate	*Size*	*Catalog No.*
Alexa Fluor® 488	50 tsts	560163
Alexa Fluor® 647	50 tests	560068
PE	50 tests	560074



Immunofluorescent staining of human breast adenocarcinoma. *MCF-7 cells (ATCC HTB-22) were cultured, fixed, permeabilized with cold methanol, stained with Alexa Fluor® 488 Mouse anti-GATA3 monoclonal antibody (pseudo-colored red, which appears pink when co-localized with the blue), and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 Bioimager System with a 20x objective and merged using BD Attovision™ software. We have observed somewhat dimmer staining when using Triton X-100 for permeabilization (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

BD Biosciences

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Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

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 using either cold methanol or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.

   Triton is a trademark of The Dow Chemical Company.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Optional blocking step: 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 conjugate 1:10 in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody conjugate, and wash the wells three times with 100 µl of 1× PBS.
8. 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.
9. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	488/10	515 LP	Fura/Fitc
BD Pathway 435	482/35	536/40	FF506

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
2. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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