Technical Data Sheet

PE-Cy™7 Mouse anti-GATA3

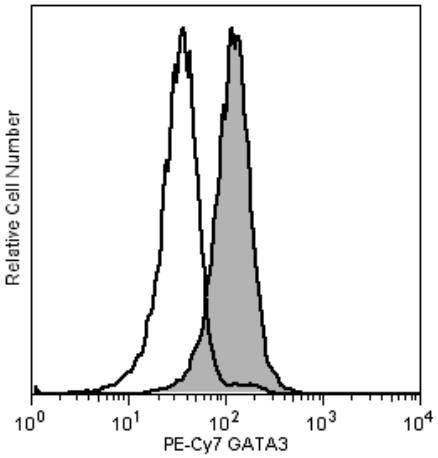
Product Information

Material Number:	**560405**
Size:	50 tests
Vol. per Test:	20 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:	QC Testing: Human Tested in Development: Mouse Predicted: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

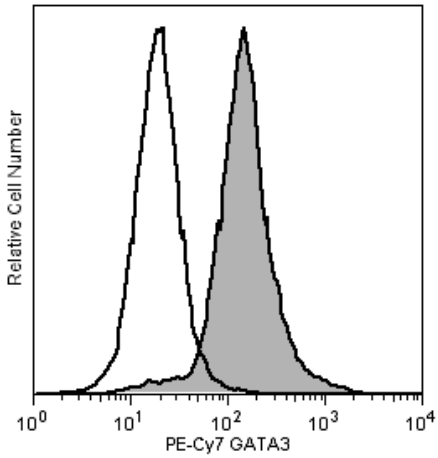
Description

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.



Comparison of GATA3 expression in human T and B cell lines. *Jurkat T leukemia (ATCC TIB152, shaded histogram) and Ramos Burkitt's lymphoma (ATCC CRL-1596, open histogram) were fixed with pre-warmed BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37ºC, permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with either PE-Cy™7 Mouse anti-GATA3 or PE-Cy™7 Mouse IgG1 κ Isotype Control (clone MOPC-21, Cat. No. 557646, not shown). The GATA3 staining on the Jurkat cell line was significantly brighter than the isotype control on Jurkat cells, while the GATA3 staining on the Ramos cells coincided very closely to its isotype control (data not shown). Thus, GATA3 expression was detected on the T cell line but not the B cell line. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*



Comparison of GATA3 expression in mouse Th2 and Th1 cell lines. *D10.G4.1 Th2 lymphoblasts (ATCC TIB-224, shaded histogram) and 2D6 Th1 clone (Ahn et al, 1998, open histogram) were fixed with pre-warmed BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37ºC, permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with either PE-Cy™7 Mouse anti-GATA3 or PE-Cy™7 Mouse IgG1 κ Isotype Control (clone MOPC-21, Cat. No. 557646, not shown). When compared to the respective isotype controls, the GATA3 staining on the D10.G4.1 cell line was significantly brighter than on the 2D6 cells. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

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

The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
557646	PE-Cy™7 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
3. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
4. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
8. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
9. 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.
10. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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