Technical Data Sheet

Mouse B Lymphocyte Activation Antibody Cocktail, with Isotype Control; PE-Cy™7 CD25, PE CD69, & FITC CD19

Product Information

Material Number:	**558064**
Size:	100 tests
Reactivity:	QC Testing: Mouse
Component:	**51-9003395**
Description:	Mouse B Lymphocyte Activation Antibody Cocktail; PE-Cy™7 CD25, PE CD69, and FITC CD19
Size:	100 tests (1 ea)
Vol. per Test:	20 ul
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-9003396**
Description:	Mouse B Lymphocyte Activation Isotype Control; PE-Cy™7, PE, and FITC
Size:	100 tests (1 ea)
Vol. per Test:	20 ul
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Mouse B Lymphocyte Activation Antibody Cocktail is a three-color reagent designed to identify major subsets of B lymphocytes by direct immunofluorescent staining with flow cytometric analysis. The PC61 antibody reacts with CD25, the low affinity IL-2 Receptor α chain (IL-2Rα, p55) expressed on activated T and B lymphocytes from all mouse strains tested. CD25 is also found on some developing B cells in the bone marrow, early developing T cells in the thymus, peripheral CD4+ regulatory T (Treg) cells, and dendritic cells. The H1.2F3 antibody reacts with CD69 (Very Early Activation antigen). Its expression is rapidly induced upon activation of lymphocytes (T, B, NK, and NK-T cells) neutrophils, and macrophages. CD69 is also expressed on thymocytes that are undergoing positive selection. The 1D3 antibody reacts with CD19, a B lymphocyte-lineage differentiation antigen that is expressed throughout B-lymphocyte development from the pro-B cell through the mature B-cell stages. Terminally differentiated plasma cells do not express CD19. The three antibodies have been titrated and pre-diluted, mixed together, and formulated for optimal staining performance. The Mouse B Lymphocyte Activation Isotype Control contains equivalent concentrations of fluorochrome- and isotype-matched negative-control immunoglobulin.

The use of three different fluorochromes for the labeling of the three different antibodies permits the recognition of each of the three antigens on each cell in a sample. The levels of expression of the three antigens distinguish the major subpopulations of developing and peripheral B lymphocytes. Additional fluorochrome-labeled reagents may be combined with the Mouse B Lymphocyte Activation Antibody Cocktail, and the Mouse B Lymphocyte Activation Isotype Control, to further characterize B-cell subpopulations.

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.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

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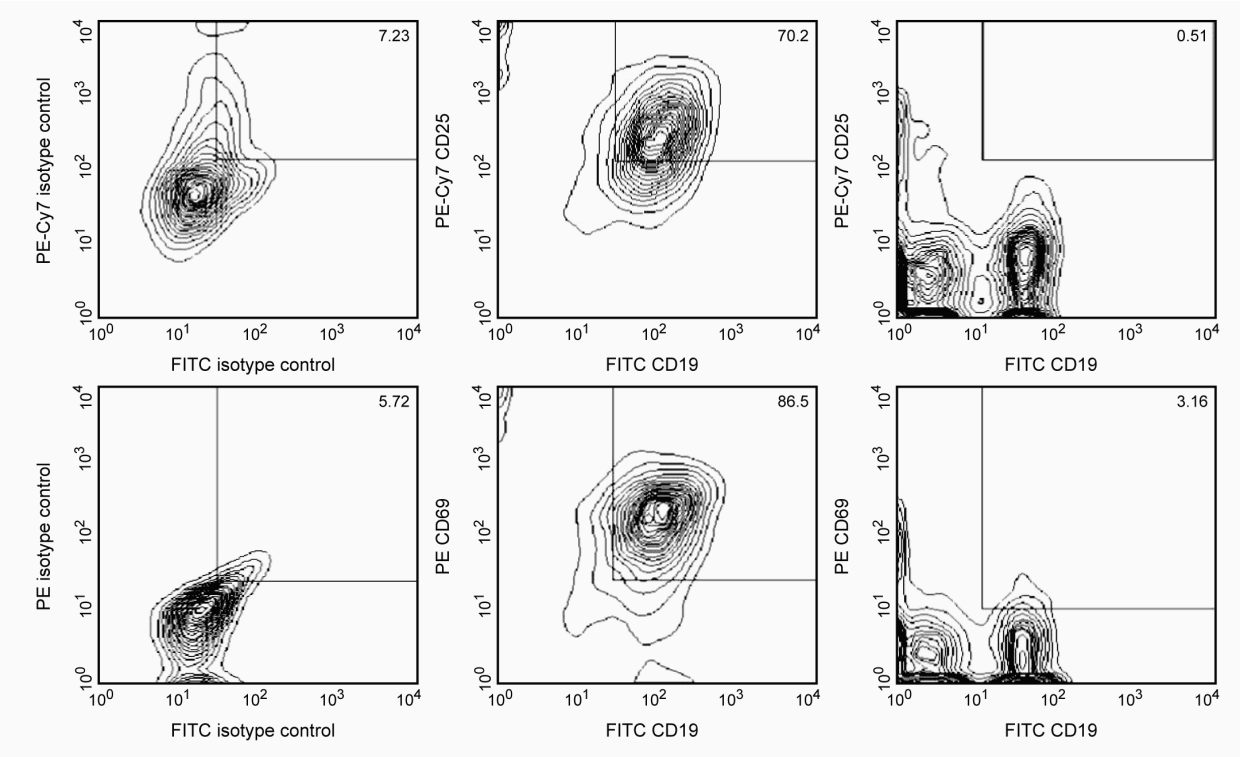

Identification of activated B lymphocytes using Mouse B Lymphocyte Activation Antibody Cocktail, with Isotype Control. BALB/c splenocytes were activated by culture for 48 hours with anti-IgM antibody (Jackson immunoresearch) and stained with either Mouse B Lymphocyte Activation Isotype Control (left panels) or Mouse B Lymphocyte Activation Antibody Cocktail (middle panels). Unactivated BALB/c splenocytes were stained with Mouse B Lymphocyte Activation Antibody Cocktail (right panels) or Mouse B Lymphocyte Activation Isotype Control (not shown). Scatter plots were used to select either activated lymphoblasts (left and middle panels) or resting lymphocytes (right panels) for data analysis. The two-color contour plots display the CD19+ B lymphocytes which express the activation antigens CD25 (top of middle and right panels) and CD69 (bottom of middle and right panels). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. 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.
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. 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).
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
10. 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.
11. 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.

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