Technical Data Sheet

Mouse B Lymphocyte Subset Antibody Cocktail, with Isotype Control; PE-Cy™7 CD45R/B220, PE CD23 (FcεRII), & APC sIgM

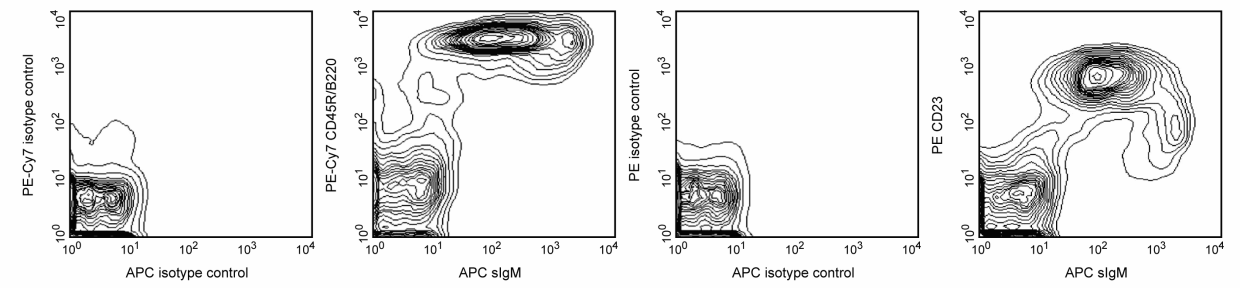
Product Information

Material Number:	**558332**
Size:	100 tests
Reactivity:	QC Testing: Mouse
Component:	**51-9000741**
Description:	Mouse B Lymphocyte Subset Antibody Cocktail; PE-Cy™7 CD45R/B220, PE CD23, and APC sIgM
Size:	100 tests (1 ea)
Vol. per Test:	20 ul
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-9000743**
Description:	Mouse B Lymphocyte Subset Isotype Control; PE-Cy™7, PE, and APC Rat IgG2a, κ
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 Subset Antibody Cocktail is a three-color reagent designed to identify major subsets of B lymphocytes by direct immunofluorescent staining with flow cytometric analysis. The RA3-6B2 antibody recognizes an epitope of the extracellular domain of CD45 that is primarily expressed, at developmentally regulated levels, on B lymphocytes at all stages from pro-B through mature, activated, antibody-secreting, and memory B cells. Although CD45R/B220 has been considered to be a defining antigen of the B-cell lineage, lytically active NK cells, some activated or apoptotic T cells, and some non-B-lineage hematopoietic progenitors have been reported to express CD45R/B220. The B3B4 antibody recognizes CD23, the low-affinity IgE Fc receptor that is expressed on mature resting conventional B cells, but not on B-1 cells (CD5+ B lymphocytes), T lymphocytes, or mast cells. The II/41 antibody recognizes the surface IgM (sIgM), specifically immunoglobulin chain, which is a component of the antigen receptor complex on immature and mature B lymphocytes, including plasma cells. The three antibodies have been titrated and pre-diluted, mixed together, and formulated for optimal staining performance. The Mouse B Lymphocyte Subset Isotype Control contains equivalent concentrations of fluorochrome- and isotype-matched negative-control immunoglobulin.

The use of three different fluorochromes for the labelling 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-labelled reagents may be combined with the Mouse B Lymphocyte Subset Antibody Cocktail, and the Mouse B Lymphocyte Subset Isotype Control, to further characterize B-cell subpopulations.



Identification of splenic B lymphocyte subsets using Mouse B Lymphocyte Subset Antibody Cocktail, with Isotype Control. *BALB/c splenocytes were stained with either Mouse B Lymphocyte Subset Isotype Control (left panels) or Mouse B Lymphocyte Subset Antibody Cocktail (right panels). The two-color contour plots display various B lymphocyte subpopulations, which can be identified by the levels of expression of CD23, CD45R/B220 and surface IgM (sIgM). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

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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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

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. 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).
7. 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.
8. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
9. 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.
10. 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.
11. 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.
12. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Stall AM, Wells SM. FACS analysis of murine B-cell populations. In: Herzenberg LA, Weir DM, Blackwell C, ed. *Weir's Handbook of Experimental Immunology.* Blackwell Science Publishers; 1997:63.1-63.17. (Biology)
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