

Technical Data Sheet

Mouse T Lymphocyte Activation Antibody Cocktail, with Isotype Control

Product Information

Material Number: 557916
Size: 100 tests
Reactivity: QC Testing: Mouse
Component: 51-9002232
Description: Mouse T Lymphocyte Activation Antibody Cocktail
Size: 100 tests (1 ea)
Vol. per Test: 20 µL
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Component: 51-9002243
Description: Mouse T Lymphocyte Activation Isotype Control
Size: 100 tests (1 ea)
Vol. per Test: 20 µl
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The Mouse T Lymphocyte Activation Antibody Cocktail is a three-color reagent designed to identify major subsets of T lymphocytes by direct immunofluorescent staining using flow cytometric analysis. This cocktail consists of the following antibody mixture: PE-Cy™7 rat anti-mouse CD25 (clone PC61), PE Armenian hamster anti-mouse CD69 (clone H1.2F3), and FITC Armenian hamster anti-mouse CD3ε (clone 145-2C11). PE-Cy™7 rat anti-mouse CD25 (clone PC61) reacts with CD25, the low-affinity IL-2 receptor α chain (IL-2Rα, p55) expressed on activated mouse T and B lymphocytes. 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. PE hamster anti-mouse CD69 (clone H1.2F3) reacts with CD69, also known as the very early activation antigen. Its expression is rapidly induced upon activation of lymphocytes (T, B, NK, and NK-T cells), neutrophils, and macrophages. CD69 has also been reported to be expressed on thymocytes that are undergoing positive selection. FITC hamster anti-mouse CD3ε (clone 145-2C11) reacts with the 25 kDa ε chain of the T-cell receptor-associated CD3 complex, expressed on thymocytes, mature T lymphocytes, and NK-T cells.

The Mouse T Lymphocyte Activation Isotype Control contains equivalent concentrations of fluorochrome- and isotype-matched negative-control immunoglobulin consisting of the following: PE-Cy™7 rat IgG1, λ (clone A110-1), PE Armenian hamster IgG1, λ (clone G235-2356) and FITC Armenian hamster IgG1, κ (clone A19-3).

Preparation and Storage

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

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

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

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

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

Application Notes

Application

Flow cytometry	Routinely Tested
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Recommended Assay Procedure:

Flow cytometry: The three antibodies contained within the Mouse T Lymphocyte Activation Antibody Cocktail have been titrated, pre-diluted, mixed together, and formulated for optimal staining performance. The use of three different fluorochromes for labeling of three different antibodies allows for the distinct recognition of three different antigens on each cell in a sample. The levels of expression of the three antigens distinguish the major subpopulations of developing and peripheral T lymphocytes. Additional fluorochrome-labeled reagents may be combined

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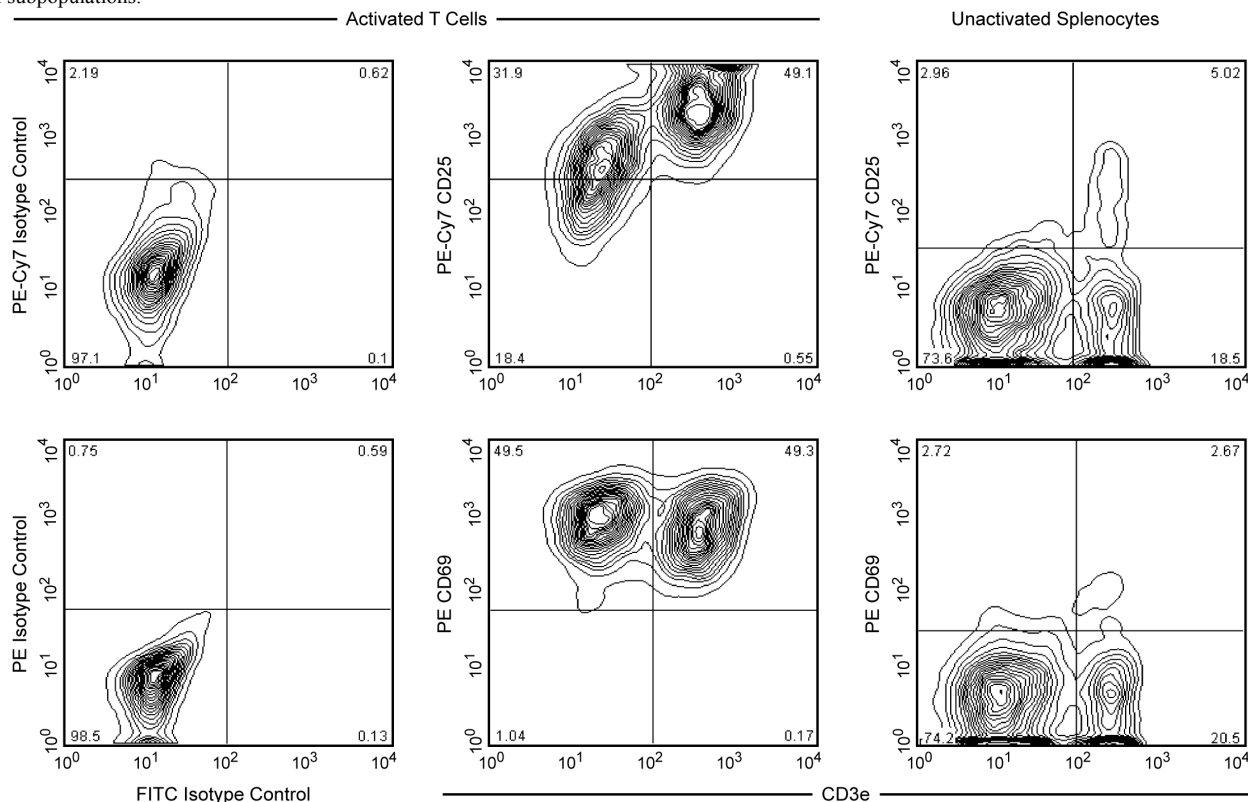
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with the Mouse T Lymphocyte Activation Antibody Cocktail, and the Mouse T Lymphocyte Activation Isotype Control, to further characterize T-cell subpopulations.



Identification of activated T-lymphocytes using the Mouse T Lymphocyte Activation Antibody Cocktail, with Isotype Control.

BALB/c splenocytes were activated in culture for 48 hours using plate-bound hamster anti-mouse CD3e antibody (clone 500A2) (Cat. No. 553238) and stained with either Mouse T Lymphocyte Activation Isotype Control (upper and lower left panels) or Mouse T Lymphocyte Activation Antibody Cocktail (upper and lower middle panels). In addition, unactivated BALB/c splenocytes were stained with the Mouse T Lymphocyte Activation Antibody Cocktail (upper and lower right panels) or the Mouse T Lymphocyte Activation Isotype Control (not shown). Scatter plots were used to select either activated lymphoblasts (left and middle panels) or resting/unactivated lymphocytes (right panels) for data analysis. The two-color contour plots display the CD3e+ T lymphocytes which express the activation antigens CD25 (upper middle and right panels) and CD69 (lower middle and right panels). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry instrument.

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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 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.
6. 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.
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. 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.
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.

References

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