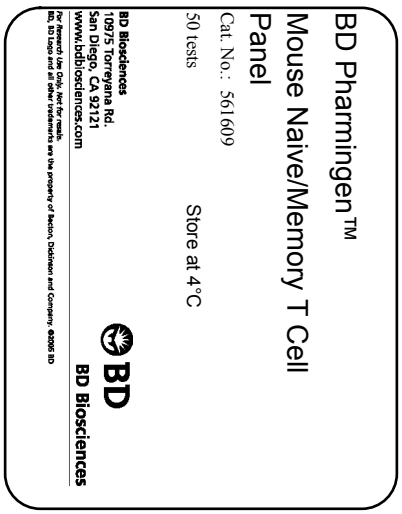

Technical Data Sheet

Mouse Naive/Memory T Cell Panel

Product Information

Material Number:	**561609**
Size:	50 tests
Reactivity:	QC Testing: Mouse

Component:	**51-9007324**
Description:	PE Rat Anti-Mouse CD44
Size:	60 tests (1 ea)
Vol. per Test:	5 µl
Clone Name:	IM7

Component:	**51-9007325**
Description:	PerCP-Cy™5.5 Rat Anti-Mouse CD4 (L3T4)
Size:	60 tests (1 ea)
Vol. per Test:	5 µl
Clone Name:	RM4-5

Component:	**51-9007326**
Description:	APC Rat Anti-Mouse CD62L
Size:	60 tests (1 ea)
Vol. per Test:	5 µl
Clone Name:	MEL-14

Component:	**51-9007327**
Description:	APC-Cy™7 Rat Anti-Mouse CD3 Molecular Complex
Size:	60 tests (1 ea)
Vol. per Test:	5 µl
Clone Name:	17A2

Description

Note: The panel is 50 test size. Extra material for each component for an additional 10 tests is provided for instrument set up purposes. The antibodies are supplied in an aqueous buffered solution containing BSA and ≤ 0.09% sodium azide.

Multicolor immunofluorescent staining followed by flow cytometric analysis and sorting has led to the phenotypic and functional characterization of multiple peripheral T cell subsets. Three major T cell subsets have been well characterized, i.e., naïve, memory and effector T cells. Naïve mouse T cells have a relatively homogenous cell surface phenotype. They express high levels of the peripheral lymph node homing receptor, CD62L (L-selectin), and low to intermediate levels of the adhesion molecule, CD44 (Pgp-1). Effector T cells coexpress variable to low levels of CD62L and high CD44 levels whereas memory T cells coexpress variable to high levels of CD62L and high levels of CD44. The Mouse Naive/Memory T Cell Panel contains fluorescent antibodies (each optimized at 5 µl per test) that are specific for the cell surface antigens: CD44, CD62L, CD4 and CD3. The panel was designed to standardize the multicolor staining and flow cytometric characterization of the three major CD4+ T cell subsets that arise as a consequence of development or clonal expansion and differentiation driven by antigenic stimulation (eg, in response to allergens, infectious disease or vaccination).
This fluorescent antibody panel was flexibly designed to permit the researcher's incorporation of FITC- or Alexa Fluor® 488-conjugated antibodies/probes or cells expressing green fluorescent proteins into flow cytometric analysis of mouse T cell subsets.

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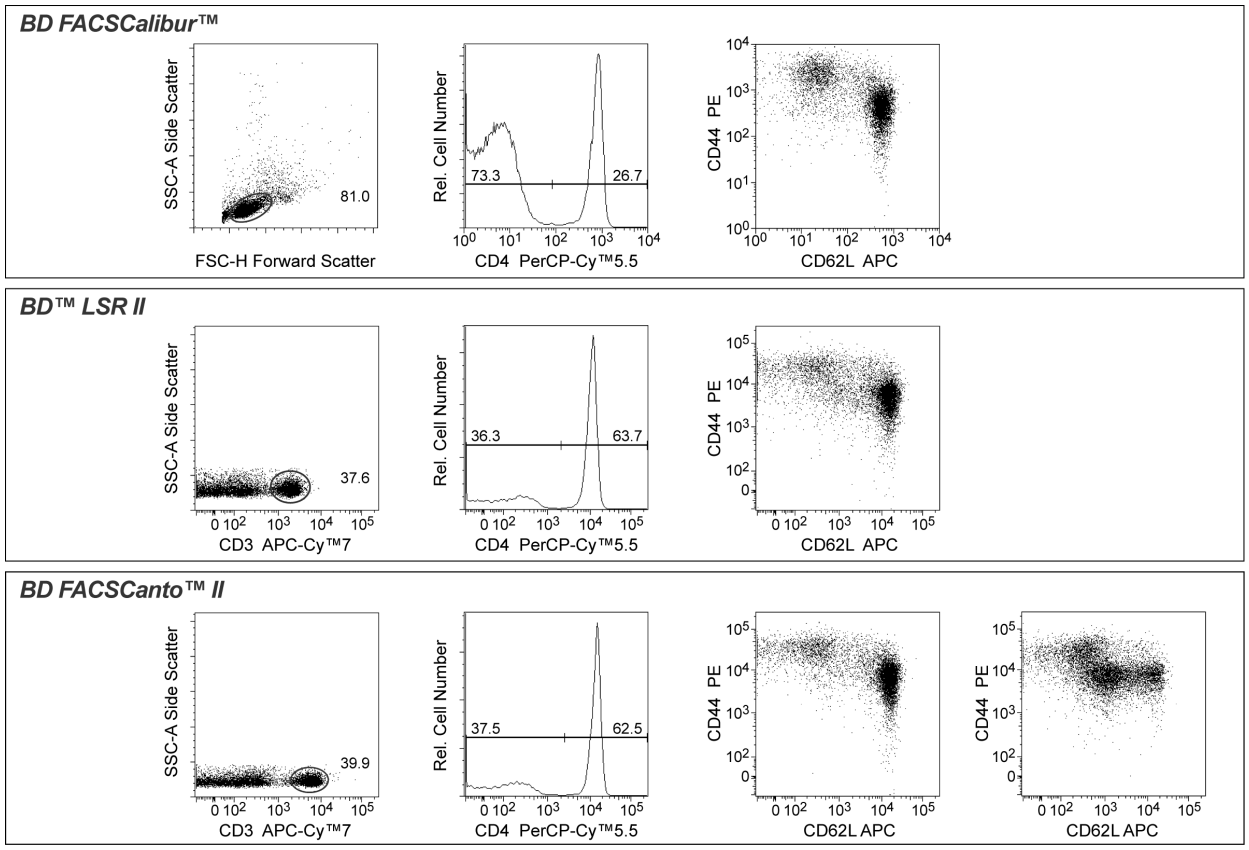

Multicolor flow cytometric analysis of naïve and memory CD4+ T cell subsets defined by the coexpressed levels of CD62L and CD44.

Splenocytes from a young (4 weeks) or older (9 weeks) BALB/c mouse donor were stained with a combination (5 µl each) of the following antibodies: PerCP-Cy™5.5 Rat Anti-Mouse CD4, PE Rat Anti-Mouse CD44, APC Rat Anti-Mouse CD62L for analysis using the FACSCalibur™ System (Top Figures) and APC-Cy™7 Rat Anti-Mouse CD3 Molecular Complex for analysis using the BD™ LSR II and FACSCanto™ II systems (Middle and Bottom Figures, respectively).

Two-color flow cytometric dot plots showing the expression of CD62L and CD44 (right dot plots) were derived from CD4+ cells (middle histograms) with the light scattering characteristics of viable lymphocytes (left FSC vs SSC dotplot from the FACSCalibur) or viable CD3+ cells (left CD3 vs SSC dotplots from the LSR II and FACSCanto II). FSC vs SSC data from multiparameter analysis using the LSR II and FACSCanto II were also used for gating viable cells (data not shown).

The patterns for the coexpressed levels of CD62L and CD44 by CD4+ T cells from the same young BALB/c mouse donor were very similar as determined by the 3 different flow cytometer systems (right dot plots). Interestingly, multicolor flow cytometric analysis of spleen cells from an older BALB/c mouse donor (far right dot plot from FACSCanto II analysis) revealed a significantly altered coexpression pattern of CD62L and CD44 by CD4+ T cells.

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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

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

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
552844	Anti-Rat Ig, κ/Negative Control (FBS) Compensation Particles Set	6.0 ml	G16-510E3
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.
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 PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
7. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
8. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
9. 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.
10. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
11. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
12. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
13. 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).
14. This conjugated product is sold under license to the following patent: US Patent No. 5,714,386.
15. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
16. PerCP is a photosynthetic accessory pigment from Glenodinium species of dinoflagellates, which is excited by the 488-nm light of an Argon ion laser and fluoresces at 675 nm. Therefore, PerCP-labelled antibodies can be used with FITC- and R-PE–labelled reagents in most single-laser flow cytometers with no significant spectral overlap of PerCP fluorescence with that of FITC or R-PE. PerCP has been reported to undergo significant photobleaching, the magnitude of which increases as laser power is increased or beam focus is narrowed. For third-color flow¬cytometric analysis using ≥25-mW laser power, we recommend PE-Cy5-, PE-Cy7–, or PerCP-Cy5.5-conjugated reagents.

References

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