Technical Data Sheet

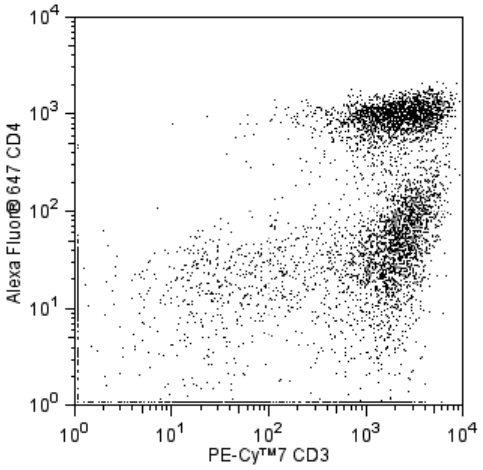
PE-Cy™7 Mouse Anti-Pig CD3ε

Product Information

Material Number:	**561477**
Alternate Name:	CD3 epsilon subunit; CD3e; T-cell surface glycoprotein CD3 epsilon chain
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	BB23-8E6-8C8
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Pig
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The BB23-8E6-8C8 monoclonal antibody specifically binds to the 25-kDa ε chain of the T-cell receptor-associated CD3 complex. It recognizes all CD4+ and most CD8+ peripheral blood T lymphocytes, most thymocytes and phytohemagglutinin-stimulated blasts, and subsets of spleen and Peyer's patch lymphocytes. BB23-8E6-8C8 is a immunoglobulin isotype switch variant of the BB23-8E6 clone. This isotype-switch variant induces a proliferative response of peripheral blood mononuclear cells. The epitope recognized by BB23-8E6 mAb was designated CD3a by the Second International Swine CD Workshop.



Multicolor flow cytometric analysis of CD3 expression on pig peripheral blood lymphocytes. *Pig whole blood was stained simultaneously with PE-Cy™7 Mouse Anti-Pig CD3ε antibody (Cat. No. 561477) and Alexa Fluor® 647 Mouse Anti-Pig CD4 antibody (Cat. No. 561472). The erythrocytes were lysed with BD PharmLyse™ Lysing Buffer (Cat. No. 555899). A two-color flow cytometric dot plot showing the correlated expression of CD3 versus CD4 was derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

PE-Cy™7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. PE-Cy™7-labeled antibodies can be used with FITC- and R-PE-labeled reagents in single-laser flow cytometers with no significant spectral overlap between PE-Cy™7 and FITC.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555899	Lysing Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
561472	Alexa Fluor® 647 Mouse Anti-Pig CD4a	50 µg	74-12-4
552868	PE-Cy™7 Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178

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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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.
4. 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.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. 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).
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. 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.
10. An isotype control should be used at the same concentration as the antibody of interest.

References

Pescovitz MD, Book BK, Aasted B. Summary of workshop findings for antibodies reacting with porcine T-cells and activation antigens: results from the Second International Swine CD Workshop. *Vet Immunol Immunopathol.* 1998; 60(3-4):251-260. (Clone-specific)
Pescovitz MD, Book BK, Aasted B. Analyses of monoclonal antibodies reacting with porcine CD3: results from the Second International Swine CD Workshop. *Vet Immunol Immunopathol.* 1998; 60(3-4):261-268. (Clone-specific)