

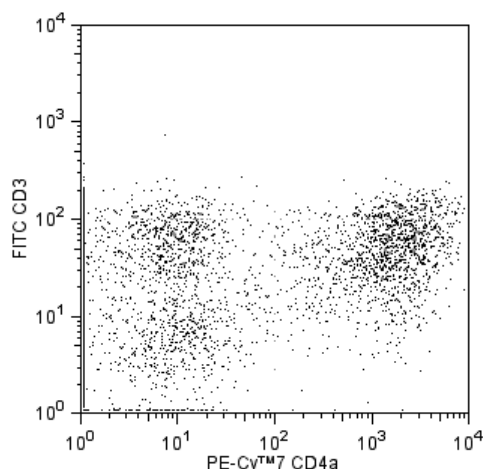
Technical Data Sheet

PE-Cy™7 Mouse Anti-Pig CD4a**Product Information**

Material Number:	561473
Alternate Name:	CD4; CD4 molecule; Lymphocyte antigen CD4
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	74-12-4
Immunogen:	dd miniature swine thymocytes
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Pig
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 74-12-4 (also known as clone PT4) monoclonal antibody specifically binds to CD4, a 55-kDa antigen expressed on T lymphocytes. This antibody does not react with CTL effectors, CTL precursors, or NK cells (ie, CD8[bright] cells) and it does not cross-react with human or bovine cells. Two peripheral T-helper lymphocyte phenotypes can be distinguished in the pig: CD4+CD8- and CD4+CD8[dull]. mAb 74-12-4 has been reported to inhibit proliferative responses of peripheral blood lymphocytes to mitogen, soluble antigen, and alloantigen. It is only marginally effective for *in vivo* depletion of peripheral CD4+ T cells. Two alloantigenic forms of CD4 have been recognized in miniature swine based upon their recognition (CD4.1) or lack of recognition (CD4.2) by mAb 74-12-4; the CD4.2 phenotype displays an autosomal recessive, non-MHC-linked, pattern of inheritance. The molecular basis for the polymorphism is a cluster of nucleotide differences leading to multiple amino-acid substitutions in the Ig CDR2-like loop structure. This mAb was clustered as anti-CD4a at the First International Swine CD Workshop. It has been reported to crossreact with chicken leukocytes.



Multicolor flow cytometric analysis of CD4 expression on pig peripheral blood lymphocytes. Pig whole blood was stained simultaneously with PE-Cy™7 Mouse Anti-Pig CD4a antibody (Cat. No. 561473) and FITC Mouse Anti-Pig CD3ε antibody (Cat. No. 559582). The erythrocytes were lysed with BD PharmLyse™ Lysing Buffer (Cat. No. 555899). Two-color flow cytometric dot plots showing the correlated expression of CD4a versus CD3 were 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
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Recommended Assay Procedure:

PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. PE-Cy7-labeled antibodies can be used with FITC- and R-PE-labeled reagents in single-laser flow cytometers with no significant spectral overlap between PE-Cy7 and FITC.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
555899	Lysing Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
559582	FITC Mouse Anti-Pig CD3ε	0.1 mg	BB23-8E6-8C8

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.

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