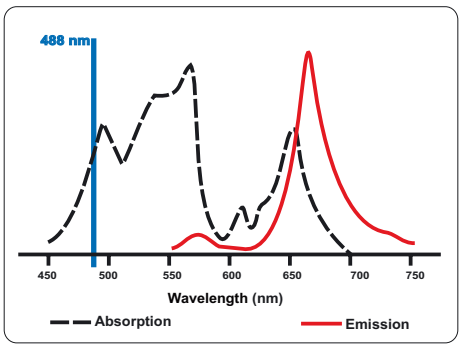


PE-Cy5

(Tandem conjugate: R-PE und Cy5)

Other designations: Tricolor[1], Cy-Chrome[2], PC5[3]

Excitation:	**488 nm**
Emission max.:	**670 nm**
Molecular weight:	**R-PE + X Cy5 >242 kDa**

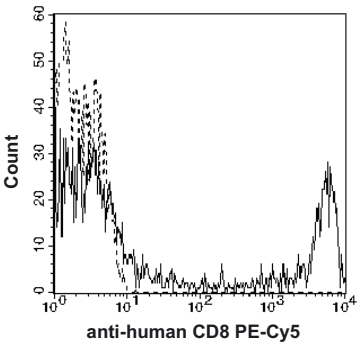
1) Caltag Laboratories 2) BD Biosciences 3) Immunotech

R-PE-Cy5

General remarks

PE-Cy5 is a tandem conjugate. The R-PE part of the tandem is excited at 488 nm and functions as an energy donor. The Cyano dye Cy5, which is inadequately excited at 488 nm, is the energy acceptor. Excitation energy is transferred from R-PE to Cy5 via energy resonance transfer. Cy5 emits the energy received in the form of long-wave light (679 nm). Since the Cy5-part of the tandem conjugates can also be directly excited at 633 nm, the use of the PE-Cy5-conjugate in conjunction with the second excitation wavelengths (488 nm; 633 nm) is only possible to a certain degree. Cyano dyes like those used in PE-Cy5, among others, can bind non-specifically under certain circumstances to monocytes. However, this characteristic of the Cyano dyes has been eliminated in tandem conjugates like Tricolor by means of special production processes. Like R-PE-conjugates, PE-tandem dyes can form agglomerations.

Flow Cytometry:



Human peripheral blood lymphocytes were marked with PE-Cy5-conjugated anti-human CD8 antibodies according to the manufacturer's instructions (Caltag). The analysis was carried out on a FACSCaliber (BD).

As a rule, PE-Cy5 can be measured with all standard commercial flow cytometers and sorters. Depending on the antibody conjugation, PE-Cy5 provides bright fluorescence signals, in comparison to the FITC conjugates. However, R-PE conjugates are preferred for antigens present at very low concentrations. PE-Cy5 is often used in combination with FITC, R-PE and PE-TR. However, since the transfer of energy from R-PE to the Cy5 is incomplete, the PE-part also emits light (ca. 5%). This “lost light” is detected on the PE-channel and makes slight compensation necessary. The Cy5-part of the tandems can be excited with a 633 nm laser (E_{max}: 670 nm). A combination of PE-Cy5 and APC (E_{max}: 660 nm) is not recommended due to the very high compensation levels with instruments lacking “cross-beam compensation”.

FC	EPICS™ XL /-MCL	Cytomics™ FC500	EPICS™ Altra	BD FACScan™	BD FACSCalibur™	BD LSR II™	BD FACSCanto™	BD Vantage™ SE	BD FACSAria™	CyAn™ MLE	CyAn™ LX	MoFlow™	CyFlow™ SL	CyFlow™ space	CyFlow™ ML	PAS™	PAS III™
Laser	488	488	488	488	488	488	488	488	488	488	488	488	488	488	488	488	488
Channel*	FI4	✓	✓	FI3	FI3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Filter optimization																	

FC: flow cytometer; * Standard filter configuration of the manufacturer

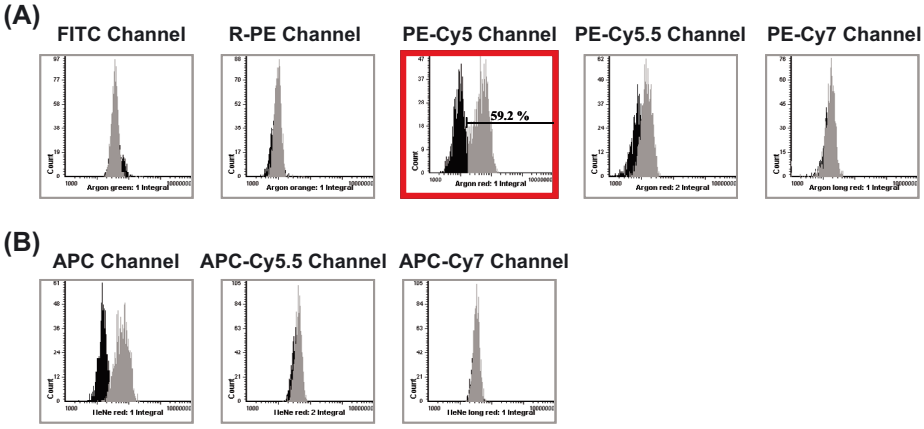
CALTAG™ Laboratories
invitrogen immunodetection

Microscopic applications

Laser Scanning Microscopy (LSM)/ Immunofluorescence (IF) Microscopy:

Tandem conjugates such as PE-Cy5 are not usually used in Laser Scanning Microscopy (LSM), as the repeated high-energy excitation causes the R-PE part of the conjugate to fade very quickly, so that the PE-fluorochrome can no longer function as an energy donor. One possibility, however, would be direct excitation of the Cyano dye Cy5 at 633 nm.
As a rule, PE-tandem conjugates are not used in Immunofluorescence Microscopy. Conventional mercury vapour lamps can directly excite the various Cyano dyes, in contrast to the monochromatic lasers (488 nm or 633 nm excitation) used in flow cytometry. A tandem construction is therefore unnecessary. For example, Cy5 conjugates or Alexa Fluor® 647-conjugates are used for the emission range around 670 nm. In this emission range, however, a corresponding camera system may be required for visualization.

Laser Scanning Cytometry (LSC):



Human leukocytes were marked with a biotinylated anti-human CD3 antibody and detected with streptavidin-PE-Cy5. Then the lymphocytes were analyzed with the LSC (CompuCyte).
The figure shows the intensity of fluorescence measured in the fluorochrome channels named above. (A): fluorochromes excitable at 488 nm. (B): fluorochromes excitable at 633 nm. Our thanks to Dr. Tarnok of the Leipzig Heart Center for making these data available.

PE-Cy5 is often used in Laser Scanning Cytometry (LSC). The incomplete transfer of energy from R-PE to the Cy5-dye results in a signal in the R-PE-channel which must be compensated. However, it can be used without difficulty in multicolor analysis. With optimized filter configurations and sequential re-analysis, PE-Cy5 can be used in combination with FITC, PE, APC, PE-Cy7, PE-Cy5.5, APC-Cy7, and APC-Cy5.5. The method and filter configuration have been described by *Lenz et al., in Proc. of SPIE, Vol. 4962, 2003*.