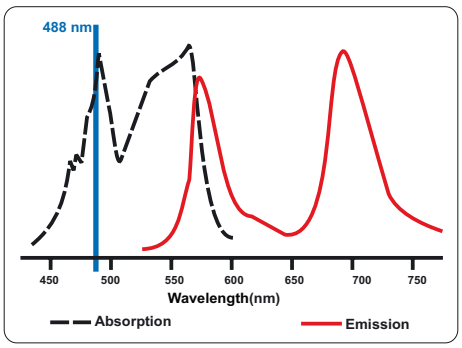


PE-Cy5.5

(tandem conjugate: R-PE and Cy5.5)

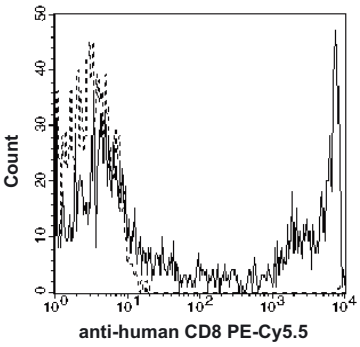
Excitation: 488 nm
Emission max.: 694 nm
Molecular weight: R-PE + X Cy5.5 >242 kDa

R-PE-Cy5.5

General remarks

The tandem conjugate PE-Cy5.5 can be excited via its R-PE component at 488 nm. Via energy resonance transfer the energy is transferred to the Cyano dye Cy5.5, which then emits this energy in the form of long-wave light (E_{max}: 694 nm). The Cyano dye Cy5.5 is only weakly excited at 633 nm. In contrast to PE-Cy5, PE-Cy5.5 can therefore be combined with APC and other fluorochromes of this excitation wavelength without difficulty. Cyano dyes such as Cy5.5 can bind non-specifically to monocytes under certain circumstances. However, special production procedures have been developed which successfully minimize this non-specific binding in the tandem conjugates.

Flow Cytometry:



Human peripheral blood lymphocytes were marked with PE-Cy5.5-conjugated anti-human CD8 antibodies according to the manufacturer's instructions (Caltag). The analysis was carried out on an FACSCaliber (BD).

The fluorochrome PE-Cy5.5 can be measured with many flow cytometers and sorters. However, filter optimization is sometimes necessary (see the table). PE-Cy5.5 offers some advantages over PE-Cy5 and PerCP for flow cytometry. It is a very bright fluorochrome that also yields good results in sorting applications (high-energy excitation). In contrast to PE-Cy5, PE-Cy5.5 can also be used without "cross-beam compensation" in combination with APC. Generally, compensation between PECy5.5 and APC is below 1% (depending on instrument and settings). Compensation with the PE-channel is possible without difficulty. When certain instruments are used, PE-Cy5.5 can be combined with FITC, R-PE, PE-Cy7, APC and APC-Cy7 among others.

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*				FI3	FI3	✓	✓	FI6	✓	✓	✓		✓	✓	✓	✓	✓
Filter optimization	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓					

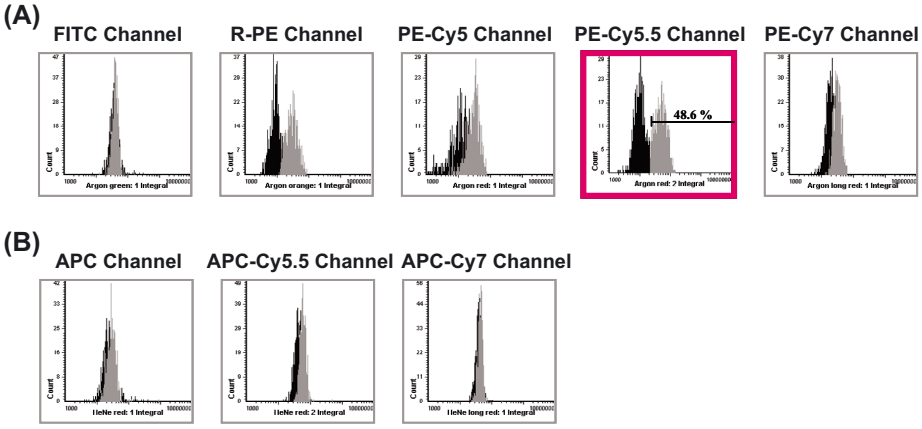
FC: flow cytometer; * Standard filter configuration of the manufacturer

Microscopic applications

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

PE-Cy5-5 is only used under certain conditions in Laser Scanning Microscopy (LSM). This is because repeated high-energy excitation quickly causes the R-PE-part of the conjugate to fade, so that it can no longer exercise its function as an energy donor.
It is unnecessary to use PE-tandem conjugates in immunofluorescence microscopy, as monochromatic light is not used here for excitation (as it is in flow cytometry or LSM) but rather white light. The Cyano dyes can therefore be excited directly. However, the intensity of fluorescence in the longwave range decreases steadily, so that Cy5.5 (E_{max}: 694 nm) would probably yield unsatisfactory results in microscopy.

Laser Scanning Cytometry (LSC):



Human leukocytes were marked with a biotinylated anti-human CD3 antibody and detected with streptavidin-PE-Cy5.5. Then the lymphocytes were analyzed on 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.

After corresponding filter adjustment, PE-Cy5.5 can also be used on the Laser Scanning Cytometer. The intensity of fluorescence is comparable to that of PE-Cy5, but lies below the signal strength of R-PE-marked antibodies. As the histograms show, compensation is required only for the R-PE and PE-Cy5 channels. Use in multicolor analysis is therefore possible. With optimized filter configurations and sequential re-analysis, PE-Cy5.5 can be used in combination with FITC, PE, APC, PE-Cy5, PE-Cy7, 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*.