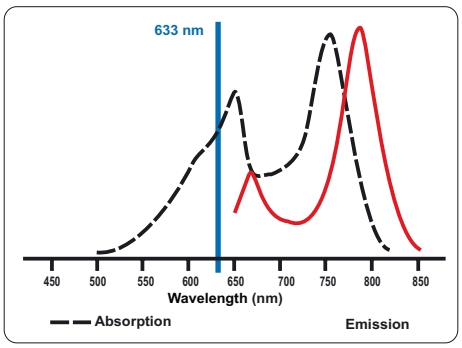


APC-Cy7

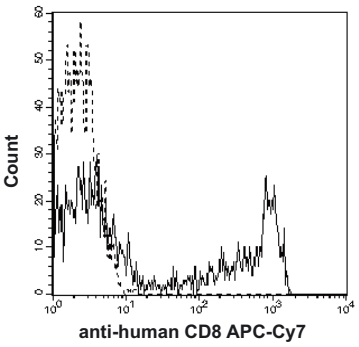
(Tandem conjugate: APC und Cy7)

Excitation: 633 nm
Emission max.: 767 nm
Molecular weight: APC + X Cy7 >105 kDa

General remarks

APC-Cy7 is an APC tandem conjugate. The APC part is excited at 633 nm. The energy of the donor (APC) is transferred to the acceptor (Cyano dye Cy7) by means of Fluorescence Resonance Energy Transfer (FRET); this acceptor in turn emits the energy received by it in the form of long-wave light (E_{max}: 767 nm). In order to avoid any possible photo-oxidation of the Cy7-part, unnecessary exposure to light should be avoided. In some instances Cy7-conjugates are also sensitive to fixing solutions containing paraformaldehyde.

Flow Cytometry:



Human peripheral blood lymphocytes were marked with APC-Cy7-conjugated anti-human CD8 antibodies according to the manufacturer's instructions (Caltag). Analysis was carried out with a modified FACScan after installation of a 633 nm laser.

The fluorochrome APC-Cy7 can be measured using flow cytometers and sorters equipped with a 633 nm laser. The signal intensity of APC-Cy7 is often lower than that of APC; this should be taken into account in the respective application. Samples marked with APC-Cy7 should be analyzed immediately after staining. This significantly reduces the possibility of light-induced oxidation of the Cy7 part. Now, however, more light-stable conjugates such as APC Alexa Fluor® 750 have also become available. Provided the necessary equipment is available, APC-Cy7 can be used with FITC, R-PE, PECy5.5, PE-Cy7 and APC without difficulty.

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*						✓	✓	FI5	✓	✓	✓		✓	✓	✓	✓	✓
Filter optimization		✓	✓									✓					

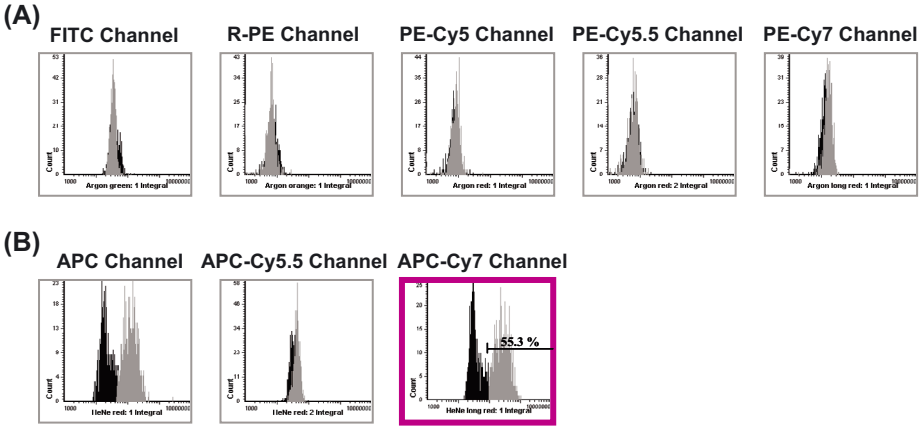
FC: Flow cytometer; * Standard filter configuration of the manufacturer

Microscopic applications

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

APC-Cy7 is hardly ever used in Laser Scanning Microscopy (LSM), as an adequate number of fluorochromes with greater photostability are available for this application. Fluorochromes in this wavelength range (767 nm) are not normally used in immunofluorescence microscopy, since the low-energy fluorescence signals (E_{max}: 767 nm) cannot be perceived by the human eye.

Laser Scanning Cytometry (LSC):



Human leukocytes were marked with a biotinylated anti-human CD3 antibody and detected with streptavidin-APC-Cy7. 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 633nm. Our thanks to Dr. Tarnok of the Leipzig Heart Center for making these data available.

APC-Cy7 can be used with the Laser Scanning Cytometer (LSC) after optimizing filter settings. However, APC-Cy7's intensity of fluorescence is below the signal intensity of APC-marked antibodies. It can be used without difficulty in multicolor analysis, since there is no cross-radiation into other fluorescence channels (R-PE, PE-Cy5, PE-Cy5.5). A signal must be compensated for only in the APC channel. With optimized filter configurations and sequential re-analysis, APC-Cy7 can be used in combination with FITC, PE, APC, PE-Cy5, PE-Cy5.5, PE-Cy7 and APC-Cy5.5. The method and filter configuration have been described by *Lenz et al., in Proc. of SPIE, Vol. 4962, 2003*.