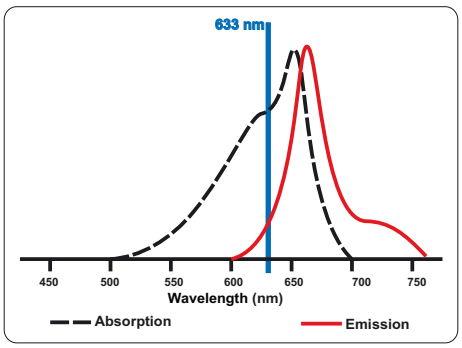


Allophycocyanin (APC)*

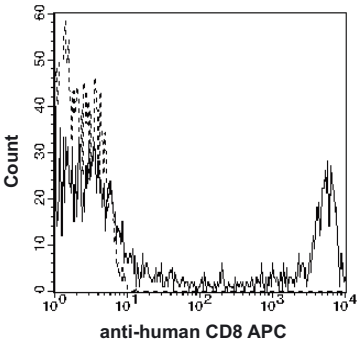
(phycobiliprotein of the cyanobacteria)

Excitation:	**633/ 635 nm**
Emission max.:	**660 nm**
Molecular weight:	**105 kDa**

General remarks

APC is an accessory photosynthesis pigment of the cyanobacteria/blue algae. It consists of a protein and 6 phycocyanobilin chromophores. APC is excited at 633/ 635 nm ("2nd laser"). It is used in various tandem conjugates such as APC-Cy5, APC-Cy5.5, and APC-Cy7 as an energy donor.

Flow Cytometry:



Human peripheral blood lymphocytes were marked with APC-conjugated anti-human CD8 antibodies as indicated by the manufacturer (Caltag). The analysis was carried out with a FACSCaliber (BD).

The fluorochrome APC can be measured on all standard commercial flow cytometers and sorters equipped with a 2nd laser (633 nm). The intensity of fluorescence is comparable to that of R-PE signals (although differences may be noticed depending on the antibody conjugation). APC is used widely in the area of multicolor flow cytometry, as it radiates only marginally into other fluorescence channels and is not excited at 488 nm ("1st laser"). When the proper instrument is used, APC can be combined with FITC, PE, PE-tandem conjugates (e.g. PE-Cy5.5, PECy7) and various APC-tandem conjugates. However, APC should not be combined with PE-Cy5 when flow cytometers without "cross-beam compensation" are used, since the spectral characteristics of the two fluorochromes require a high degree of 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	633	633	633	633	633	633	633	633	633	633	633	633	633	633	633	633	633
Channel*		✓	✓		FI4	✓	✓	FI4 (2)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Filter optimization																	

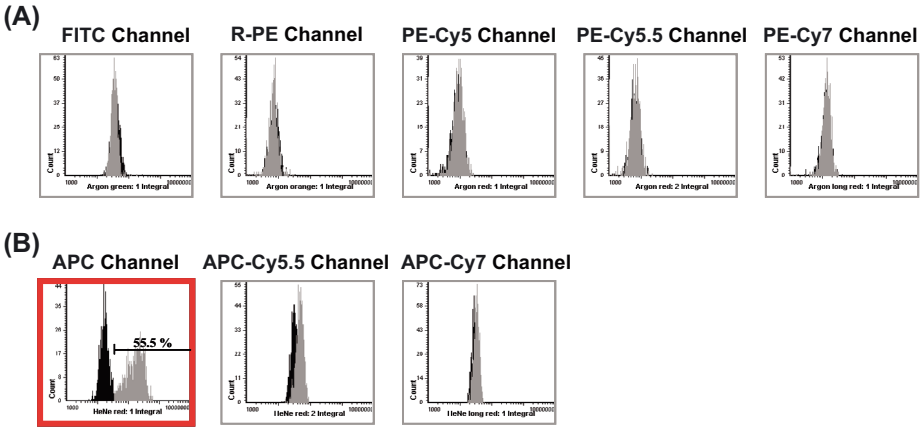
FC: flow cytometer; * Standard filter configuration of the manufacturer

Microscopic applications

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

Given the proper instrument configuration, APC can also be used in Laser Scanning Microscopy (LSM). However, the use of Cy5 conjugates or Alexa Fluor® 647 conjugates is more common.
When APC is used in immunofluorescence microscopy, it must be remembered that the sensitivity of the human eye diminishes for wavelengths above 650 nm. Under certain circumstances, the use of corresponding camera systems may be required to visualize and detect fluorochromes with a maximum emission of more than 650 nm (e.g. APC).

Laser Scanning Cytometry (LSC):



Human leukocytes were marked with a biotinylated anti-human CD3 antibody and detected with streptavidin-APC. 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.

Given the proper laser equipment (633 nm), APC can be used without difficulty in Laser Scanning Cytometry (LSC). This fluorochrome is often used in multicolor analysis since it provides bright signals and radiates only marginally into the other fluorescence channels. The literature describes the use of APC in combination with FITC, PE, PE-Cy5, PE-Cy7, PE-Cy5.5, APC-Cy7 and APC-Cy5.5 (*Lenz et al., Proc. of SPIE Vol. 4962, 2003*).