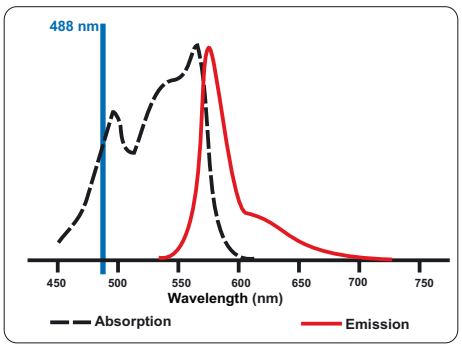


R-Phycoerythrin (R-PE)*

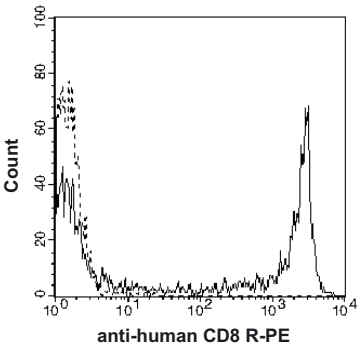
(phycobiliprotein of red algae)

Excitation:	**488 nm**
Emission max.:	**575 nm**
Molecular weight:	**> 240 kDa**

General remarks

Phycoerythrin (R-PE) is a constituent part of the photosynthesis apparatus of red algae. It consists of a protein and 34 phycoerythrobilin fluorochromes. This fluorochrome/protein ratio is the main reason for the bright intensity of R-PE. However, the low degree of photostability limits its range of application. Its high molecular weight and its tendency to agglomeration must be taken into consideration during the use of R-PE conjugates. Non-specific binding of the fluorochrome to cells is occasionally observed. R-PE-conjugated antibodies should not be stored at -20 °C, as freezing damages the fluorochrome.

Flow Cytometry:



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

R-PE can be measured on all standard commercial flow cytometers and sorters. Its high light intensity makes it the fluorochrome of choice in the detection of antigens expressed in low concentrations. The R-PE fluorochrome is also often used in multicolor analysis. However, the spectral characteristics of R-PE require compensation with FITC (E_{max}: 525 nm) and fluorochromes in the long-wave emission range (E_{max}: 550 to ca. 680 nm). For intracellular stainings the size of the fluorochrome should be weighed against its bright intensity. Under certain circumstances it may be advantageous to use Alexa Fluor® conjugates, or FITC conjugates for intracellular applications.

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*	FI2	✓	FI2	FI2	FI2	✓	✓	FI2	✓	✓	✓	✓	✓	✓	✓	✓	✓
Filter optimization																	

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

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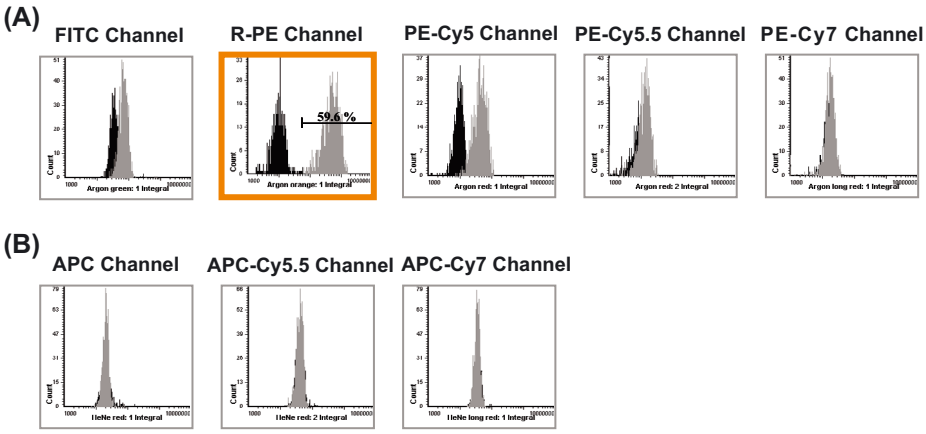
Microscopic applications

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

R-PE-conjugated antibodies are not usually used in Laser Scanning Microscopy (LSM), as the repeated high-energy excitation by the laser causes the dye to fade quickly.
Similarly, R-PE is not often used in immunofluorescence microscopy. Under certain circumstances, special covering media can delay the photo bleaching of R-PE and improve the results. Generally, however, other red dyes are used in IF such as Cyano dye Cy3 (E_{max}: 565 nm) or Alexa fluorchromes.

Laser Scanning Cytometry (LSC):



Human leukocytes were marked with a biotinylated anti-human CD3 antibody and detected with streptavidin-R-PE. 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. (The method has been described by *Lenz et al., Proc. of SPIEVol. 4962, 2003*). Our thanks to Dr. Tarnok of the Leipzig Heart Center for making these data available.

R-PE is regularly used in Laser Scanning Cytometry (LSC). Its high intensity of fluorescence permits the detection of antigens that are expressed in low concentrations. Depending on the instrument used, however, the signal intensity is somewhat less than that generated on the flow cytometer. As the above histograms show, some parts of the R-PE signal are also detectable on the FITC and PE-Cy5 channels. This makes compensation necessary. However, use in multicolor analysis is possible without difficulty.