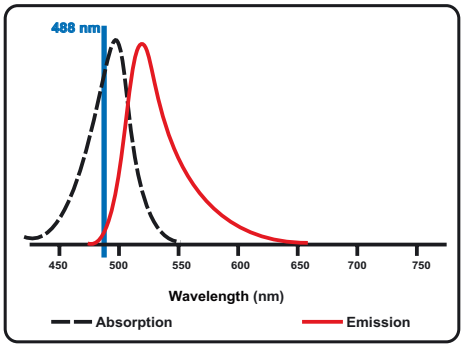


Fluorescein isothiocyanat (FITC)

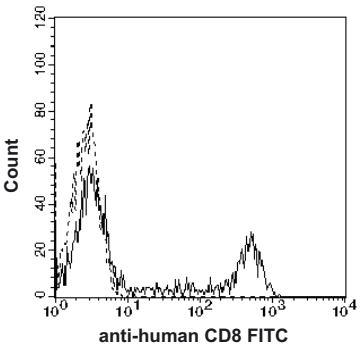
(Xanthene dye)

Excitation:	**488 nm**
Emission max.:	**525 nm**
Molecular weight:	**389 Da**

General remarks

FITC has a good quantum yield. Almost half of the absorbed photons are emitted in the form of fluorescent light. Compared to Alexa Fluor® 488, a fluorochrome with similar spectral characteristics, however, FITC exhibits weaker intensity of fluorescence and less photostability. The intensity of fluorescence of the fluorochrome FITC is also affected by the pH value. These characteristics must be taken into account in each respective application.

Flow Cytometry:



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

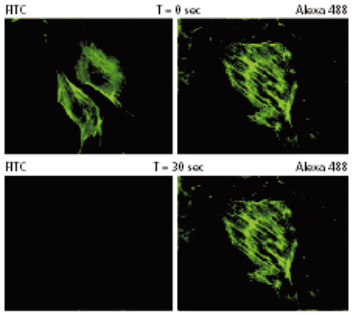
FITC is widely used in the area of flow cytometry. This fluorochrome can usually be measured with any standard commercial flow cytometer and sorter. Compensation between FITC and other fluorochromes needs to be taken into consideration only in the case of phycoerythrin (E_{max}: 575 nm) due to the overlapping emission spectra. However, compensation of the two fluorochromes is usually unproblematic. FITC is therefore often used in multicolor flow cytometry. The low fluorescent intensity of the FITC fluorochrome should be taken into account when considering its usage. It is a good idea to detect strongly expressed antigens with FITC-conjugated antibodies and to demonstrate the presence of weakly expressed markers with the help of stronger fluorochromes such as Alexa Fluor® 488 or phycoerythrin (R-PE, E_{max}: 575nm).

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*	FI1	✓	FI1	FI1	FI1	✓	✓	FI1	✓	✓	✓	✓	✓	✓	✓	✓	✓
Filter optimization																	

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

Microscopic applications

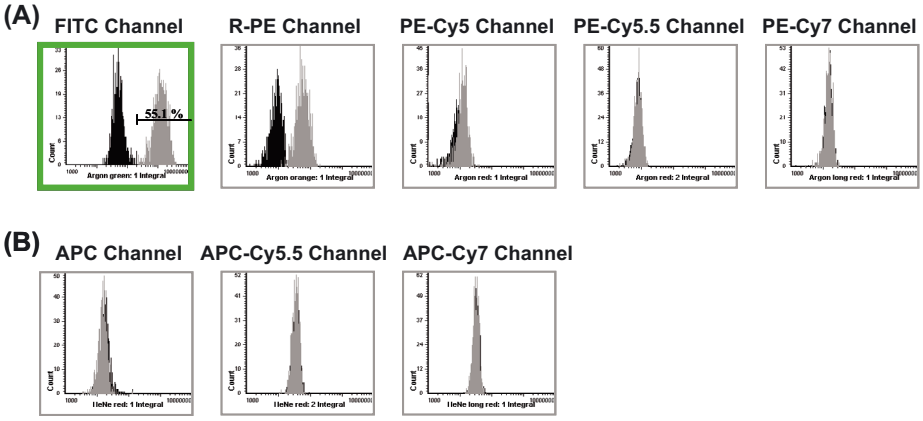
Laser Scanning Microscopy (LSM)/ Immunofluorescence (IF) Microscopy:



Actin filaments of bovine endothelial cells were marked with FITC or Alexa Fluor® 488-coupled phalloidin. After exposure to light for 30 seconds, FITC has a residual intensity of ca. 20%, in contrast to the fluorochrome Alexa Fluor® 488, which is nearly photostable. (Figure from Molecular Probes, Eugene, OR)

FITC-conjugated antibodies are used very widely in Laser Scanning Microscopy (LSM) and in Immunofluorescence (IF) Microscopy. However, the low photostability of the fluorochrome FITC becomes evident in these applications. In particular, high-energy excitation with a laser (LSM) results in rapid photo bleaching of the FITC fluorochrome. With special covering media, however, results can be notably improved. Nowadays more photostable alternatives to the fluorochrome FITC are available, such as Alexa Fluor® 488 or the Cyano dye Cy2.

Laser Scanning Cytometry (LSC):



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

FITC is often used in Laser Scanning Cytometry. The spectral characteristics of the FITC fluorochrome cause it to cross-radiate only into the R-PE channel. This must be taken into account accordingly. The use of FITC-conjugated antibodies in multicolor-analysis is therefore possible without difficulty. The intensity of the FITC fluorochrome is sufficient for the detection of antigens expressed at high concentrations. Reanalysis of FITC-marked samples is nearly impossible due to the low degree of photostability. However, photo bleaching of the samples can be delayed by the use of proper covering media.