Pre-adsorbed affinity purified antibodies
Secondary antibodies, although affinity purified, may cross-react with serum proteins from other species beside the reaction with the original IgG used for immunization and purification. In some cases this cross-reactivity is unwanted and a more specific antibody is required. The cross-reactivity is reduced by removing the undesired cross-reactive antibodies during a pre-adsorption step on an affinity column, which has been loaded with serum proteins from selected species. The specificity of this pre-adsorbed antibody is then checked against IgG's from different species in an ELISA to guarantee that the pre-adsorbed antibody has minimum cross-reactivity with the stated species. This selectivity is indicated by an annotation following the name of the antibody : i.e. Min x Rt indicates a minimal binding of the relevant antibody to rat serum proteins. All our antibodies are available unconjugated or covalently coupled to a variety of labels (see below).

Technical tip

Whole IgG : these antibodies suit classical immunodetection applications (ELISA, Blotting), and immunocapture applications (immunoprecipitation, coating).

F(ab')$_2$: these antibodies can be used for applications where Fc fragments can cause non-specific binding, i.e. to cells Ig receptors.

Preadsorbed antibodies : these antibodies are particularly useful when cross-reactivity is feared especially in multiple labeling applications

FluoProbes® labels

Welcome to our new range of fluorophores called FluoProbes®. Among all the FluoProbes® dyes that were developed, we have selected 4 for immunoglobulin labeling according to :

- Superior fluorscence properties
- Execellent stability (to ptt, light)
- Stokes's shift from 15 to 180 nm
- Cover the whole spectra
- Suit most common light sources and standard filters

TRITC-labeled pallido-ALa neurons (C), and FluoProbes488-labeled ENK+ terminals (*D*), using confocal laser scanning microscopy. The location of the BDA+ perikarya in *C* is shown by *asterisk*

Dye	$\lambda_{abs.}$	$\lambda_{em.}$	Suitable light source	Suitable filter	Remarks
FP488	493 nm	518 nm	Argon ion Laser	FITC/Cy2 filter	Superior brightness to Fluoresceins. Ultimate photostability
FP546	545 nm	561 nm	Kr/Ar ion Laser He-Ne laser mercury lamp	TRITC or Cy3 filter	Paired with FP642, Fluoresceins
FP494	494 nm	628 nm	Argon ion Laser	FP494 compatible filter	
FP642	642 nm	660 nm	Kr/Ar ion laser He-Ne laser	Cy5 filter	Lower autofluorescence if biological specimens. Paired with a variety of other fluorophores (incl.FP642)

*TRITC is also known as Tetramethyl Rhodamine Isothiocyanate; it is available as item #FP-47004 and other derivatives.

*SR101 is also known as Sulforhodamine101. It is available as item #FP-47006, and other derivatives as FP-AM402 (SR101-PEO-SE).

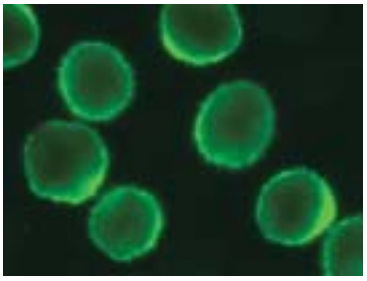
*RRX is a derivate of Lissamine rhodamine B, also known as Rhodamine Red-X™ (a trademark from MolecularProbes). It is available as item #FP-R1404 and other derivatives.

*Cy™ is a trademark from Amresham

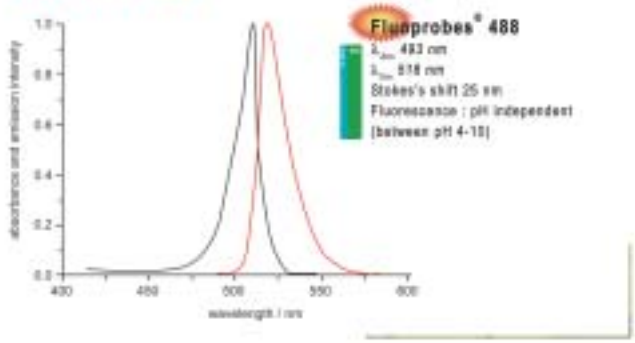
Fluorescent labels

Technical informations about fluorophores and antibodies

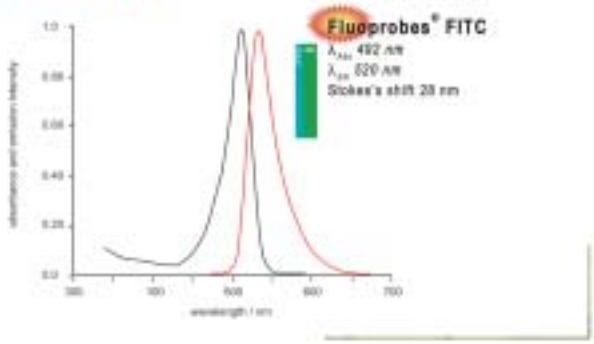
Spectra of IgG conjugated with FluoProbes® dyes



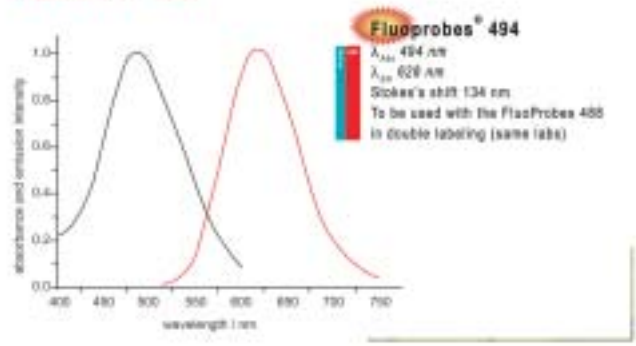
FluoProbes® 488



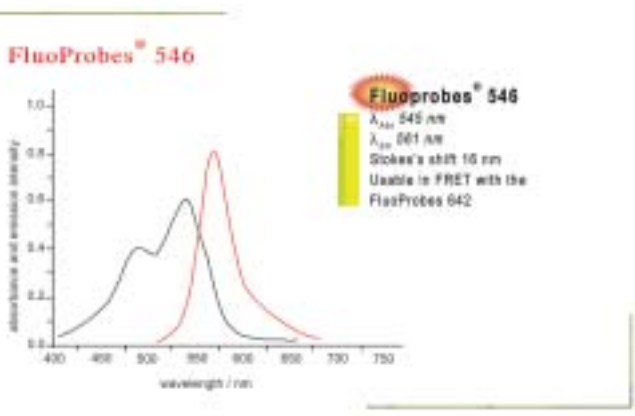
FluoProbes® FITC



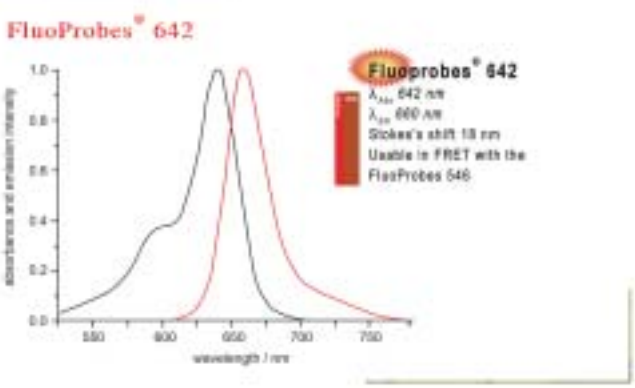
FluoProbes® 494



FluoProbes® 546



FluoProbes® 642



Benefits of FluoProbes® conjugates

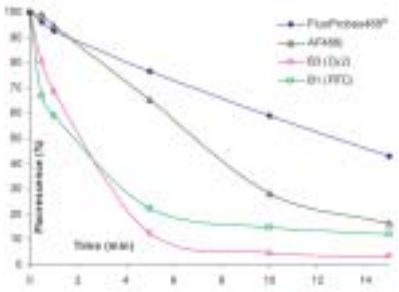
- Brightness* :
  FluoProbes® dyes show an enhanced fluorescence compared to other similar conjugates.
- Photostability :
  Our conjugates are more photostable than most other conjugates allowing longer exposure for image capture.
- Color choice :
  FluoProbes® dyes range in color from green to red.
- Ready to use :
  All FluoProbes® conjugates are available in ready-to use solution, allowing researchers to save time and money.
- Species-Specificity :
  The secondary antibodies conjugated to our FluoProbes® dyes have higher specificities, exhibiting a very high Signal/Noise ratio. It is then possible to have very species-specific secondary antibodies conjugated with the best fluorochromes actually available on the market.

* Brightness
Brightness is a crucial feature because it finally determines the signal of detection. It is related to :
- Energy input during light excitation of the fluorochrome (appreciated with light output of the instrument and Molar absorption parameter of the Fluorochrome)
- Percentage of energy transfer in light emissions (quantum yield)
- Measurement performance (instrument sensitivity)
- Fluorochrome/antibody coupling ratio.

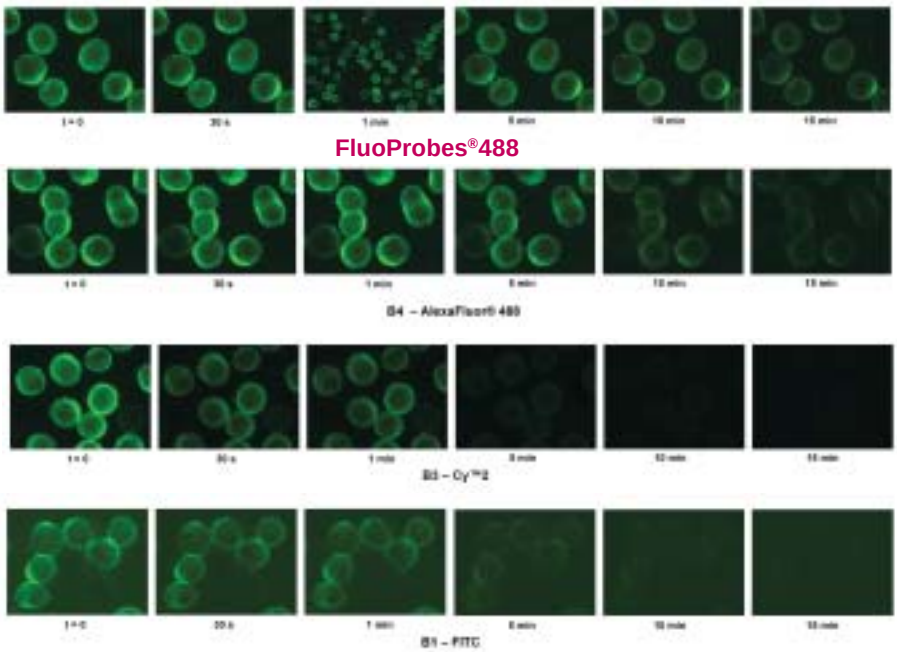
FluoProbes® fluorochromes have high Molar Absorption and quantum yield to maximize brightness for demanding techniques, as confocal microscopy. Additionally, the coupling ratio of Dye per Protein (D/P) has been optimized to ensure optimal sensitivity.

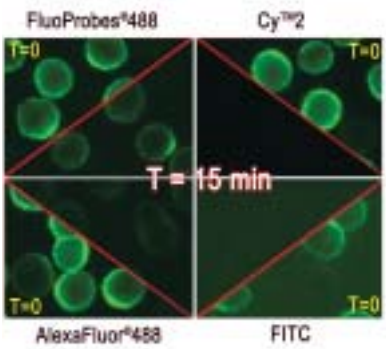
Technical tip

Photostability
Fluorescence fading raises important limitations of use, i.e. during direct observation of anatomy by fluorescent microscopy, the declining fluorescence strains eyes and demands to occlude the beam and wait fluorescence restoration. More decidedly, fading ruins the benefits of advanced microscope imaging systems, as digital camera and confocal microscopy : fluorescence is recorded during long periods to acquire more photons and thus increase the signal (provided background stays low), or to allow autofluorescence to fade, or because the sample is illuminated over a long scanning time.



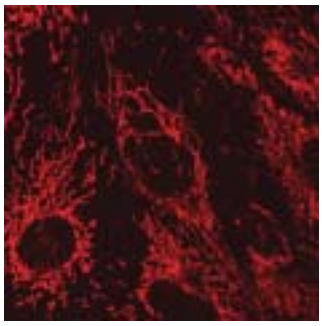
FluoProbes®488 demonstrates superior photostability in a confocal microscopy experiment of fluorescence fading in comparison with competitor labels.
Myc tagged CHO cells were labeled by a biotinylated anti myc Ab followed by green fluorescent streptavidins, then imaged with a Nikon TE2000E equiped with FITC filter Chroma 41001 for 800ms (FluoProbes®488), or 170 ms (others) at times 0, 30 sec, 5 min, 10 min and 15 min during light exposure. After quantitation of signal on 10 RBCs, normalized fluorescence is plotted against light illumination time.





Immunologicals - Secondary reagents

Fluorescent labels



FluoProbes®642 labeling of Mitochondria from CV1 cell line.

Excellent Brightness
Brightness is a crucial feature because it finally determines the signal of detection. It is related to :

- Energy input during light excitation of the fluorochrome (appreciated with light output of the instrument and Molar absorption parameter of the Fluorochrome)
- Percentage of energy transfert in light emissions (quantum yield)
- Measurement performance (instrument sensitivity)
- Fluorochrome/antibody coupling ratio.

FluoProbes® fluorochromes have high Molar Absorption and quantum yield to maximize brightness in demanding techniques, as confocal microscopy. Additionally, the coupling ratio of Dye per Protein (D/P) has been optimized to ensure optimal sensitivity, for each dye.

Stability of light emission (with pH, light...)
An ideal dye for immunoanalysis should usually emit light in a stable manner. However, fluorescence is a complex process affected by many environment factors : for example, fluorescence of Fluorescein is largely affected by pH and environment hydrophilicity, and it decreases upon continuous light illumination (fading).

To that consern, FluoProbes® fluorochromes 488, 494, 546, 642 have very stable fluorescence. They are independent to pH and light exposure. Also, FluoProbes®-Ab linking chemistry ensures excellent storage stability.

TRITC (Tetramethyl Rhodamine Isothiocyanate) ; it is available as item #FP-47004A and other derivatives.

SR101 (Sulforhodamine101). It is available as item #FP-47006A, and other derivatives as FP-AM4020 (SR101-PEO-SE).

RRX is a derivate of Lissamine rhodamine B

* % are % of maximum excitation

Note : Most of these labels are available as building block and reactive derivates for synthesis and labeling, as well as conjugated to serum proteins (i.e. used as controls) and specific probes. Custom labeling is also possible (please refer to the Custom service section).

Conventional fluorophores

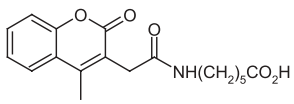
Conventional labels include the followings :

Dye	$\lambda_{exc.}$	$\lambda_{em.}$	Suitable light source *	Suitable filter set	Remarks
AMCA	350 nm	450 nm	mercury lampwater-cooled argon ion laser	UV filter	Not for single labeling
FITC	492 nm	520 nm	Argon ion Laser	FITC/Cy2 filter	
FP488	493 nm	518 nm	Argon ion Laser	FITC/Cy2 filter	Superior brightness to Fluoresceins Ultimate photostability
Cy™2	492 nm	516 nm	Argon ion Laser	FITC/Cy2 filter	
FP546	545 nm	561 nm	Kr/Ar ion Laser He-Ne laser mercury lamp Kr/Ar ion Laser (50% max) He-Ne laser (543 nm line) mercury lamp (546 nm line)	TRITC or Cy3 filter	Paired with FP642, Fluoresceins
Cy™3	550 nm	570 nm		TRITC or Cy3 filter	Paired with Cy5 (CLSM/KrAr) Not recommended with Fluorescein
TRITC	550 nm	570 nm	Kr/Ar ion Laser	TRITC or Cy3 filter	
RRX	570 nm	590 nm	Kr/Ar ion Laser		
SR101	596 nm	620 nm	krypton laser	620 ± 10-nm bandpass filter	
FP494	494 nm	628 nm	Argon ion Laser	FP494 compatible filter	
FP642	642 nm	660 nm	Kr/Ar ion laser He-Ne laser	Cy5 filter	Benefit of lower autofluorescence if biological specimens Paired with a with a variety of other fluorophores (incl.FP642)
Cy™5	650 nm	670 nm	Kr/Ar ion laser (98%max) He-Ne laser (63%/633 nm line)	Cy5 filter	Benefit of lower autofluorescence if biological specimens Paired with a with a variety of other fluorophores Not suited for conventional epifluorescence microscopes

Aminomethylcoumarin Acetate (AMCA)

AMCA is an excellent dye for multiple labeling, since there is minimal fluorescence overlap with fluorescein and little or no overlap with longer-wavelength-emitting fluorophores. Applications for multiple labeling with this probe include both immunohistochemistry and flow cytometry.

Since blue fluorescence is more difficult for the human eye to be seen than other colors, AMCA should be used with the most abundant antigens in multiple-labeling experiments. Other ways to improve the visibility of AMCA include dark adapting the eyes, using fluorite instead of glass objectives, not mounting sections in media that may absorb some of the UV light (such as plastic-based media), and by photography since colour films usually have good sensitivity to blue light. AMCA also tends to fade as rapidly as fluorescein and therefore may require the use of an anti-fading agent such as n-propyl gallate.
Alternatively, FluoProbes®390A may be an interesting alternative because it is more photostable, gives a wider dynamic range, and penetrates more easily cell structures.

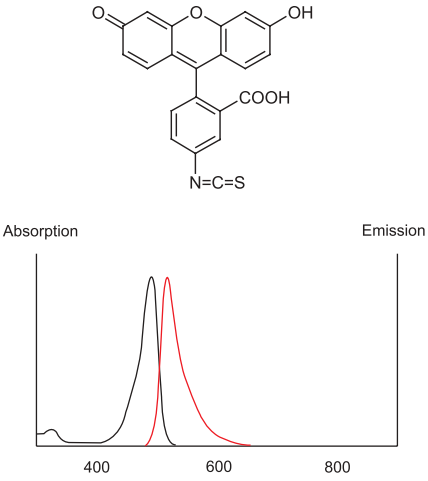


See AMCA absorption and emission fluorescence spectra page A322.
AMCA is available as item FP-AZ393A and FP-AM364A (MTS-SSR101X) for labelings.

FITC (Fluorescein)

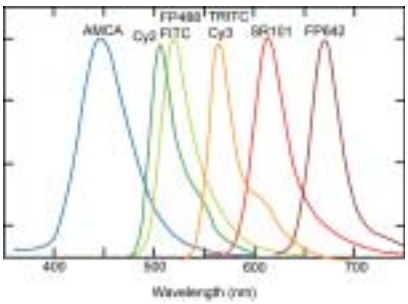
Fluorescein is the most popular fluorochrome since the first ages of fluorescence technologies. It absorbs light maximally at 492 nm and fluoresces at 520 nm, with one of the highest yield amongst conventional dyes. The fluoresceine Isothiocyanate derivative (FITC) succinimidyl ester (#FP-40295A) has become the standard derivate to label antibodies. However, FITC has some drawbacks, including pH sensitivity and photobleaching, that renders it not suitable for demanding applications (Confocal microscopy, Polarization fluorescence, microarray,...). Anti fading agents have been used to overcome photobleaching, but signal and sensitivity are lowered. Several other dyes have been proposed. For most applications, we recommend our alternative green label, the FluoProbes®488, that shows superior fluorescence, lower background, pH insensitivity, and unsurpassed photostability (see page A319). However, we continue to provide FITC-labeled secondary Abs to those customers who can't change of label or for specific applications (pH probes in cells). We use a highly purified FITC isoform 5 to label our high quality antibodies.

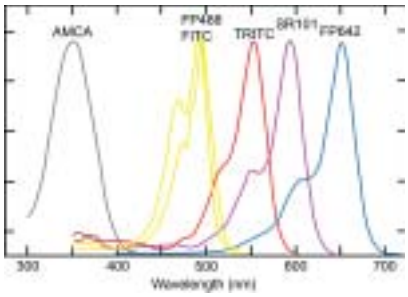
Although FITC has been used most commonly with TRITC for double labeling, better color separation is achieved by using Rhodamine Red-X or SR101 (Titus et al., J. Immunol. Methods. 1982. 50, 193), or FluoProbes®647. However, the use of SR101 may lead to slightly higher background staining (Wessendorf and Brelje, Histochemistry. 1992. 98, 81). For double labeling in flow cytometry, phycoerythrin (instead of Rhodamine) conjugates (see page A348) are recommended for use with FITC since both can be excited by the same light wavelength (488 nm).
Fluorescein derivatives are also available for labeling, as item FP-01739K (FITC), FP-M1299A (FAM-X-SE), and many others.



Absorption and emission spectra of 5-FITC (#FP-01739K).

Fluorescent labels





Excitation spectrum (normalized)
Emission spectrum (normalized).

*TRITC (Tetramethyl Rhodamine Isothiocyanate)
*SR101 (Sulforhodamine101)
*RRX is a derivate of Lissamine rhodamine B

Excitation spectrum (normalized)
Emission spectrum (normalized).

TRITC, SR101, and RRX*

These 3 rhodamine based dyes cover the orange to red range, with good fluorescence properties. They are used separately, or combined (TRITC / SR101), or more often paired to green labels (i.e. Fluoresceins), and IR labels (i.e. TRITC / FluoProbes®647)

Although TRITC* has been used more often with FITC for double labeling, better color separation is achieved by using RRX* or SR101* (Titus et al., J. Immunol. Methods. 1982. 50, 193). However, the use of SR101 may lead to slightly higher background staining (Wessendorf and Brelje, Histochemistry. 1992. 98, 81). For double labeling in flow cytometry, phycoerythrin (instead of Rhodamine) conjugates are recommended for use with FITC since both can be excited by a single wavelength (488 nm) of light. In several techniques with adapted filter set, FluoProbes®494 represents an interesting pair with FITC, giving higher photostability, better signal linearity and better membrane permeability. TRITC is available as labeling agent FP-47004 A for custom labeling.

RRX* is significantly brighter than those conjugated with Lissamine rhodamine B, replacing advantagely TRITC. RRX is particularly recommended for triple labeling using a confocal laser scanning microscope along with FP488 (FITC) and FP647 (Cy5).

SR101* is a good red fluorescent dye for microscopy. The conjugates are very bright and extremely stable (resistant to proteases). With SR101 (FP-47006), an improved version SR101-PEO-SE (FP-AM402A) is also available for custom labeling.

Cyanine dyes (Cy™2, Cy™3, Cy™5)

Cyanine-structure based dyes were introduced to cover more uniformaly the light spectrum, offering additionally some advantages over former fluorochromes. Although many structures modifications were proposed, most cyanine dyes self aggregate in aqueous solution or show cis-trans isomerization that decrease the fluorescence. Cy™2 (green), Cy™3 (orange) and Cy™5 (red) were the most popularized, until new dyes were proposed. For instance FluoProbes®488 shows advantages with higher signal, lower noise, and better photostability. Now, Cy™3 and Cy™5 remain standards in some labs, notably for microarrays. Alternatives are FluoProbes®488, 546, and 642, with higher performances (improved sensitivity) in most applications.

*Cy™ is a trademark from Amersham