FT-FP488c(FluoProbes488-Comparison)

FluoProbes®488

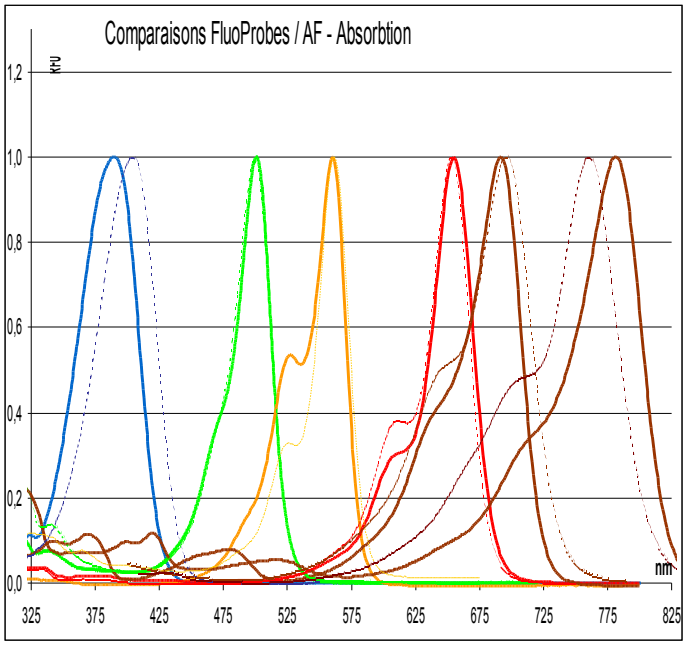
This technical sheet presents comparative datas and applications of **FluoProbes488 dye**, that is the most popularly used green label, matching conventional fluorophores and competitors dyes. These highlight its superior features (brightness, photostability,...)

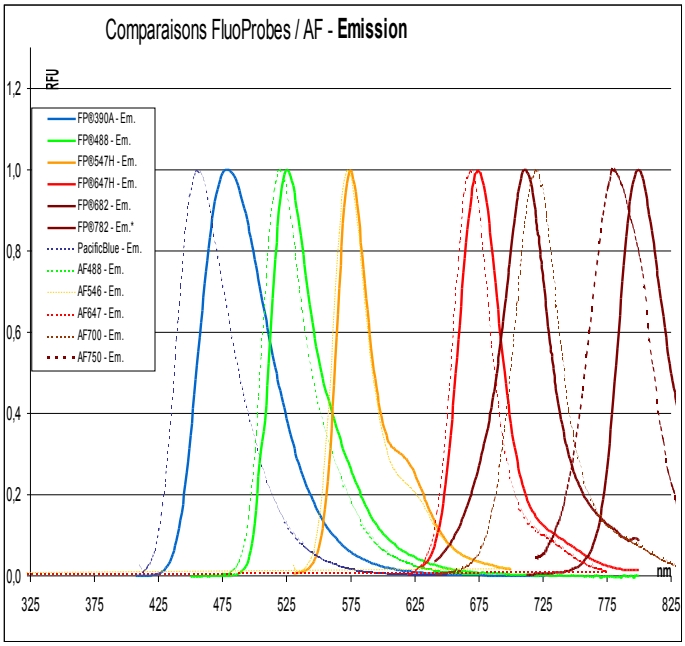
For more information, and information about other FluoProbes labels, please ask FluoProbes at info@fluoprobes.com.

Fluorescence characteristics

Product name cat.number	λ_{exc}\λ_{em} max. (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Comments FluoProbes benefits / *Conventionnal dyes limitations*
FluoProbes® 488	493 / 519	85 000	• Bright green fluorescence, compatible with standard filters for FITC/ Cy™2 • pH-independent fluorescence between pH 5 and 9 • Ultimate photostability, hence minimal fading • Superior alternative to FITC, A488 • Ideal for confocal microscopy, but suits also any other microscopy or technique including microplate readers and FCM.
FITC	494 / 515	70 000	• photostability is unsuficient with pH, and upon continuous illumination
Cy™2	489 / 506	150 000	• brighter than FITC, not photostable
A488	494 / 517	71 000	• very bright, photostable, expensive

Abs. & Fluorescence Spectra





FluoProbes488 use by FCM & Microscopy

compared **by CFSM** (confocal microscopy) with competitor labels:

FP488 demonstrates superior photostability: 15 min photostability whereas competitors have only 1-2min photostability (see below). FP488 produces also brighter images (see below), and has been used in mulitplex combined to blue, orange, red and IR emitting dyes (see below 3-color experiment with Hoechst and FP505).

compared **by flow cytometry (FCM)**:

FP488 has a **superior signal and lower background**. It can be used alternatively to conventional dyes (FITC, DTAF) ad competitor dyes (A488, Cy2). I.e. +30% higher signal than FITC was shown analysing RBCs with labeled streptavidins using a BD Facs scan. See also comparisons of AnnexinV-FP488 to AnnexinV-FITC (NT-BH9390).

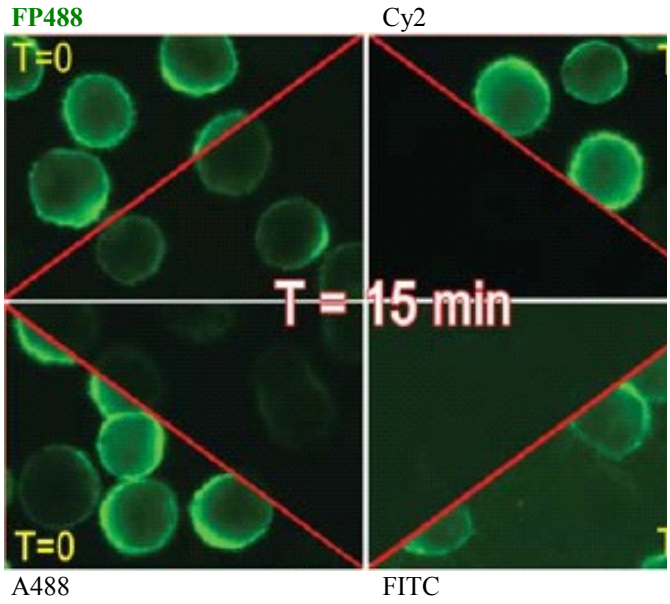
: Streptavidin labeled to detect red blood cells with BD Facscan. FP488 has a signal superior to FITC (+30%) and lower signal (not shown).

FluoProbes488 superior photostability

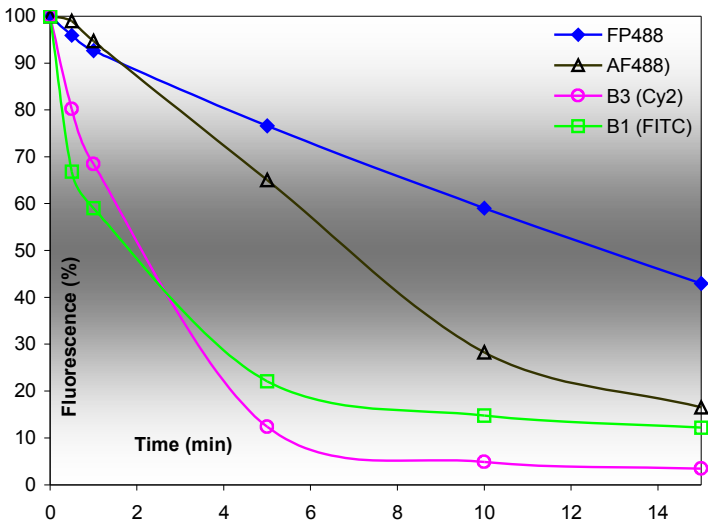
FluoProbes®488 demonstrates superior photostability compared by CFSM (confocal microscopy) with competitor labels: it shows incredible 15 min lasting signal upon continuous illumination whereas competitors fade within 1-2min photostability. A superior signal is also shown.

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 at times 0, 30sec, 5min, 10min and 15min durations of light exposure. After quantitation of the signal, normalized fluorescence is plotted against light illumination time.

High and lasting signal to noise of FluoProbes488



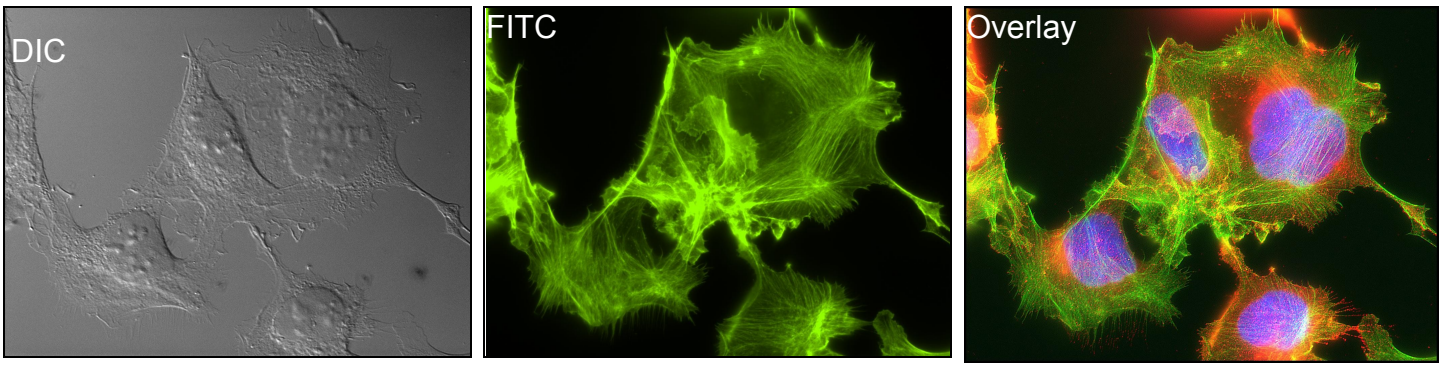
Ultimate photostability of FluoProbes488 (FP488)



Material & methods – CFSM Microscopy:
Nikon TE200E
Exfo W-cite light source-no attenuation
Camera: cooled CCD digital camera Roper CoolSnap HQ
Exposure time: 170 msec
Digitization: 12 bits (levels of gray 0-4095, full scale)

CHO-KiSS-1 receptor, Myc taggedd – fixed on polylysine coated slides, mounted in 50% PBS 50% glycerol.
Anti myc clone $9^{E}10$ at 10µg/ml
Streptavidin-FluoProbes488 (or labeled by competoto dyes AF488, V2, FITC) 2µg/ml
Filter set: Chroma 41001 (for FITC… Ex 480/40, DM 505, Em 535/50)

FluoProbes488 great brightness in Microscopy



HEK-293-Myc-GPR54 cells stained with anti myc tag mouse antibody then with anti mouse IgG labeled by FP547H, Phalloidin-FP505 and Hoechst33342. DIC: observed by differential interference contrast; FITC: observed with a FITC filter; Overlay= merge of images with FITC, Hoechst and TexasRed filters

Technical tip: Photostability
Fluorescence fading raises important limitations of use, i.e. during direct observation of anatomy and pathology by fluorescent microscopy, the declining fluorescence strains eyes and demands to oclude the beam and wait fluorescence restoration (switch off pas ok). More decidely, fading ruins the benefits of advanced microscope imaging systems, as digital camera and confocal microscopy: the fluorescence is recorded during important times to acquire more photons and thus increase the signal (provided background stays low), or to allow autofluorescence to fade (especially with visible light), or because the sample is illuminated over a long lasting scanning.

FluoProbes Greeen dyes Applications

NT-FP647c **Using Annexin-FP488 in Confocal Microscopy application**.
AnnexinV-FP488 greatly detects apoptotic Jurkat cells, multiplexed to GPR54 detection with FP647H.

NT-FP547c **Using Phalloiding-FP505 in Confocal Microscopy application in 3-color experiment**.
The cytoskeletton of HEK293 cells are vizualized, including stress fiber due to apoptosis, multiplexed to GPR54 detection with FP547H and the nucleus detected using Hoechst33342.

Other applications
Use of FP488 dye for **microArrays** – FP488 excellent phtostability with excellent brightnesare taken to good account in production of diagnostic arrays - InoMust serology multiplexed diagnostic. Inquire
Use of FP488 dye for **polarisation anisotropy fluorescence** – FP488 is one of the rare dyes with a negative value of anisotropy. Inquire
Comparison of FP488 in Confocal Microscopy. FP488 labeled Secondary antibody is compared to A488 in double staining with Cy5 – No crosstalk. Inquire
Using secondary Abs labeled by FP488 in Confocal Microscopy - insulin secretory granules. American Society for Biochemistry and Molecular Biology ; Brunner ; Inquire
Using Annexin-FP488 in Confocal Microscopy application - ischemia/reperfusion injured mouse heart ; Shows superior quantum yield and improved stability compared with FITC, A488; yields a 1:1 stoichiometric complex with the reporter group Inquire

Other information

Other FluorProbes dyes: see FT-FPlist
Other products: please consult the BioSciences catalogue
Catalog size quantities and prices may be found at http://www.interchim.com/ .
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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