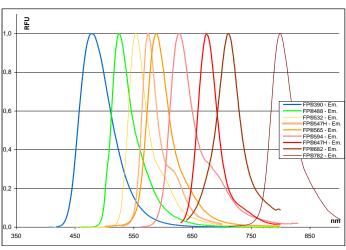
FluoProbes® Labels

Fluoprobes® dyes are designed for labeling biomolecules in advanced fluorescent detection techniques. Applications include multiple labeling, FRET, Quenching, polarisation anisotropy fluorescence, and life time resolved fluorescence, with protein as well as with nucleic acids. Fluoprobes® fluorophores are available as different derivatives for conjugation with conventional chemistry methods. This technical sheet presents the main features of selected standard FluoProbes dyes, that are the most popularly used in fluorescence analysis.

For more information, and information about other FluoProbes labels, please search at www.interchim.com/interchim/customers/ or ask FluoProbes at info@fluoprobes.com.



Selected standard FluoProbes® fluorescent labels for labeling biomolecules.



Product name	MW £	$\lambda_{\rm exc} \setminus \lambda_{\rm em}$. max.	mol. abs.	Comments (key features – see below for more info)
cat.number	(g·mol⁻¹)	(nm)	$(M^{-1}cm^{-1})$	
FluoProbes®390A	343.4	390 / 479	24 000	Bright blue fluorescence Large stocke's shift A good alternative to AMCA
<u>FluoProbes® 488</u>	804	493 / 519	85 000	 Bright green fluorescence, compatible with std filters for FITC/Cy2 Unrivalled stability (photo-stability upon continuous illumination, hence minimal fading, at PHs, for storage) Superior alternative to FITC, Cy2, A488 Ideal for confocal microscopy, suits also any other techniques
FluoProbes® 532A	765	532 / 553	115 000	 Compatible with standard filters for A532 Very bright yellow fluorescence Ideal using the frequency-doubled Nd:YAG laser.
FluoProbes® 547H	735.8	557 / 574	150 000	 Bright orange fluorescence, compatible with standard filters for Cy3, A546, Rhodamine TRITC High brightness
FluoProbes® 565A	611	563 / 592	120 000	Bright orange, compatible with standard filters for A568
FluoProbes® 594	1078	591 / 617	92 000	Bright red fluorescence, compatible with standard filters for TR Outperforms SR101/TR
FluoProbes® 647H	761.3	653 / 674	250 000	 Very bright far red fluorescence, compatible with standard filters for Cy5, A647 – outperforms notably in FCM Very high brightness and photostability
FluoProbes® 682	853	690 / 709	140 000	Bright IR fluorescence – enhanced solubility Compatible with standard filters for Cy5.5, IRD700, A680
FluoProbes® 752	879	748 / 772	270 000	 Very bright IR fluorescence Compatible with standard filters for Cy out performs the expensive dye previously used for oligos lab
FluoProbes® 782	976.1	783 / 800	170 000	Double negatively charged Soluble in MetOH, EtOH,; DMF, DMSO
Other remarkable Fluoprobes labels				FP415 (blue), FP465A (blue/green), FP505 (green/yellow), FP532A (Orange), FP565A(Orangeed), FP594A (far red, XXL), FP®633A(red), FluoProbes® XXL(long stock's shift)

^{£:} MW of free acid

See derivatives by NHS(<u>BA6800</u>), Maleimide (<u>BA6810</u>), Azide (<u>YE4970</u>), Alkyne (<u>YE4970</u>), Hydrazide (1Q7081), Amine (BU4960), Carboxyl (BU4960)





Scientific and technical Information - Labels

Fluoprobes®390A label:

- **Bright blue fluorescence** (λ_{exc} ./ $\lambda_{\text{em.}}$: 390 / 479nm)
- excited efficiently in the range 360 410 nm, with a maximum at 390nm. A useful excitation source is, e.g. a Mercury Arc Lamp with its lines at 365nm & 405nm, or violet 4005 laser.
- good extinction coefficient (ε at λmax.: 24 000 M⁻¹cm⁻¹) and high quantum yield (QY>90%).
- high stability at physiological pH-values.
- large stokes shift (89nm) reduces background noise caused by scattered light.
- Its NHS-ester and maleimide show excellent solubility in polar solvents like DMF, DMSO or acetonitrile, the dye itself is moderately hydrophilic, that (when coupled) limits occurring of self-quenching, even at high ratios.
- compatible with standard filters for AMCA

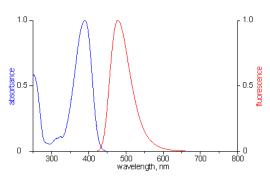
Derivative	FluoProbes®390A
-COOH	FP-BS5610
-NHS	FP-BS5620
-MAL	FP-BS5630
-HYD	FP-FI3340
-SAV	FP-BM7700
Lab.kit	FP-CG5390

Fluoprobes®488 label:

- Bright green fluorescence (λ_{exc} ./ $\lambda_{em.}$: 593 / 519nm): High extinction coefficient (ϵ at λ max.: 95 000 M⁻¹cm⁻¹) and quantum yield (QY>80%).
- Fair hydrophilicity allows for higher coupling ratios, minimal self-quenching and lower background.
- **pH-independent fluorescence** between pH 4 and 10
- Ultimate photostability upon light exposure (see figure):
 Fading is mininal, longer integration of signal in digital imaging can be achieved allowing to detect low abundance molecules withour signal amplification methods; re-analysis of samples remain quantitative; you do not need to use antifading agents or additives (reduce price, time, toxicity)
- Compatible with standard filters for FITC/CyTM2...
- Excited by Argon488 blue-green laser

Derivative FluoProbes®488		
-COOH	FP-BA6790	
-NHS	FP-BA6800	
-MAL	FP-BA6810	
-HYDR	FP-B38820	
-SAV	FP-BA2221	
-Label.kit	FP-BE3750	

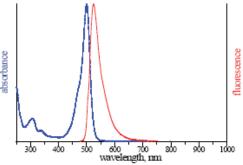
Inquire for conjugates (II Antibodies, AnnexinV,...)



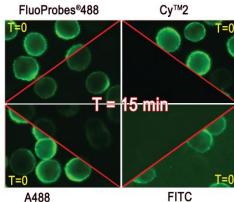
Absorption and emission spectra

As a result, FluoProbes®390 is:

- a superior alternative to AMCA (λ_{exc} ./ $\lambda_{em.}$: 354/442nm, EC:19 000)
- **ideal for radiography**, but suits also any other microscopy or other fluorescent techniques.
- ± Fluoprobes® labels are available derivatized with a carboxylic group (COOH), an amino group (NH2), Succinimidyl ester (NHS), Maleimide (MAL), Hydrazide (HYD), streptavidn (SAV), and other functional groups (Alkyne,...), ligands (phalloidins,...) as well as protein labeling kits. Inquire



- FP[®]488 is brighter and much more photostable than FITC, Cy2 and others. FP488 is the most photostable of any tested dye, incl. A488.
- FP[®]488 is **ideal for confocal microscopy**, but suits also any other microscopy or technique including microplate readers and FCM.



More information

Superior photostability:

FluoProbes®

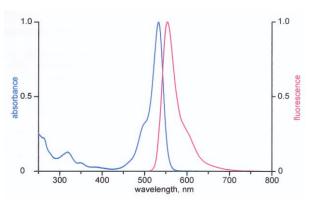
FT-FPstd_(StandardSelectedFluoProbesLabels)

Fluoprobes®532A label:

- very bright orange fluorescence (λ_{exc}./λ_{em.}: 532 / 553nm; EC: 115 000; QY:90%) excited most efficiently in the range 515 - 545 nm, i.e. by the 532nm line of the frequency-doubled Nd:YAG laser. Excellent extinction coefficient and quantum yield.
- Excellent water solubility
- Net electrical charge of -1
- Compatible with standard filters for A532

More information

Derivative	FluoProbes®532A
-COOH	FP-BA6940
-NHS	FP-BA6950
-MAL	FP-BA6950
-HYDR	FP-BA6960
-SAV	FP-CA5590



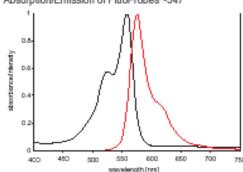
Absorption and emission spectra (in water)

Fluoprobes® labels are available derivatized with a carboxylic group (COOH), an amino group (NH2), Succinimidyl ester (NHS), Maleimide (MAL), Hydrazide (HYD), streptavidin (SAV), and other functional groups (Alkyne, ...), ligands (phalloidins, ...) as well as protein labeling kits. Inquire

Fluoprobes®547H label:

- Bright orange-red fluorescence $(\lambda_{\rm exc}./\lambda_{\rm em.}: 557 / 574 nm)$
- High brightness, FP®547H shows elevated extinction coefficient and can usually be coupled at high ratios without quenching. One achieve higher signal and lower background than with other standard orange fluorophores (TMR, A546), and reduce the fading observed in some applications with Rhodamine TRITC, Cy3 labels.
- pH-independent fluorescence and photostable
- Compatible with standard filters for TRITC/CyTM3...
- Net negatively charged, and good solubility in water*

Absorption/Emission of FluoProbes®-547



As a result, FluoProbes®547H is:

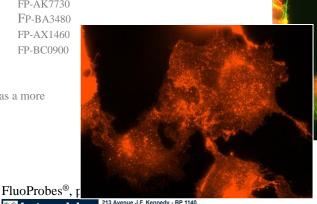
- a superior alternative to TRITC fluorophores, Cy3 and A555/546.
- It suits any fluorescent techniques: microscopy fluorescence (including confocal microscopy), microplate and microarray assays, FCM...

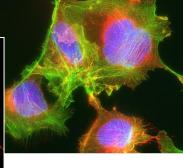
Superior brightness and photostability (to Cy3, A546/555) Great 3-color imaging (Hoechst, FP488, FP547H):

Derivative FluoProbes®547H FluoProbes®547 -COOH FP-BX5020 FP-BA3460 $-NH_2$ FP-BX5030 FP-BA3470 -NHS FP-BX8920 FP-AK7730 -MAL FP-CB1000 FP-BA3480 -SAV FP-CA5570 FP-AX1460 label.kit FP-BZ9600 FP-BC0900

More information

*FluoProbes547H is also available as a more polar and less hydrophilic version, the original FP547 dye.





Info@fluoprobes.com Technical-support@fluoprobes.com Order-online@fluoprobes.com

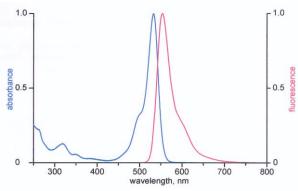


Fluoprobes®565A label:

- very bright red fluorescence (λ_{exc}./λ_{em.}: 563 / 592nm; EC: 120 000; QY:90%)
- **photostable** (much superior to Cy3)
- compatible with TR filters
- Net electrical charge of -1

More information

Derivative	FluoProbes®56
-COOH	FP-BA7030
-NHS	FP-BA7040
-MAL	FP-BA7050
-SAV	FP-CA5610



Absorption and emission spectra

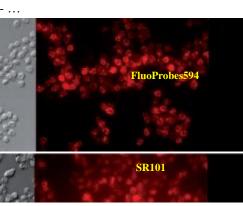
Fluoprobes® labels are available derivatized with a carboxylic group (COOH), an amino group (NH2), Succinimidyl ester (NHS), Maleimide (MAL), Hydrazide (HYD), streptavidn (SAV), and other functional groups (Alkyne, ...), ligands (phalloidins, ...) as well as protein labeling kits. Inquire

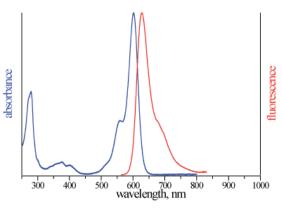
Fluoprobes®594 label:

- **bright dark red fluorescence** (λ_{exc}./λ_{em.}: 591/617nm; EC: 92 000)
- FP594 is soluble in water, methanol, DMF
- FP594 is excited optimally at 591 nm.
- FP594 emits optimally at 617nm, compatible with standard filters for TR

More information

Derivative	FluoProbes®594
-COOH	FP-FO7600
-NH2	FP-FO7590
-NHS	FP-FO3340
-TFP	FP-DY2610
-MAL	FP-FJ0800
-HYD	FP-FJ0800
-SAV	FP-FV8180





Absorption and emission spectra

FluoProbes®594A is

- a superior alternative to TR

(**SulfoRhodamine101:** 595-605/615nm, FP-83766A)

- suits any fluorescence technique (microarrays, microscopy, FCM, ...).

Anti CD59 labeled RBCs immunotaining, with controls by phase contrast (left) and IF without primary antibody (right)
FluoProbes594 (upper) shows brighter signal with lower background than TR (SulfoRhodamine101).



Fluoprobes®647H label:

- Bright Far Red fluorescence (λ_{exc.}/λ_{em.}: 653 / 674nm; EC: 250 000)
- High brightness:
 FP®647 shows elevated extinction coefficient and can usually be coupled at high ratios without quenching. One achieve higher signal and lower background than with other standard red fluorophores (Cy5), and reduce the fading observed in some applications.
- pH-independent fluorescence and photostable
- Compatible with standard filters for CyTM5, A647...
- Net negatively charged, and good solubility in water*
- Can be combined in multiplex with FP488, FP547H
 Can be combined in tandem with Cy7 (λ_{ex.}/λ_{em.}:
 488/782nm) and in tandem with APC (ex./em.: 595-640/782nm)

More information

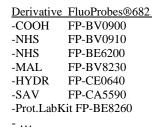
FluoProbes547H is available with many derivatisations*

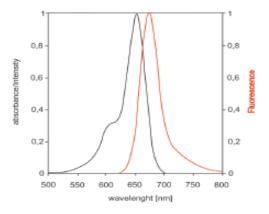
Derivative	FluoProbes®647H	FluoProbes®647*
-COOH	FP-CD9830	FP-BA3830
$-NH_2$	FP-BX5040	FP-BA3840
-NHS	FP-BZ8810	FP-AK7740
-MAL	FP-CB1010	FP-AZ5280
-HYD		FP-BP5530
-SAV	FP-CA5640	FP-BA1270
Lab.Kit	FP-BZ9610	FP-BA0310

^{*} FP647H is also available as a more polar and less hydrophilic version, the original $\underline{FP647}$ dye.

Fluoprobes®682 label:

- Bright IR solid state fluorescences (λ_{exc}./λ_{em}.: 690/709nm; EC: 140 000)
- Hig molar extinction (140 000M⁻¹cm⁻¹)
- Soluble in water, methanol, DMF, DMSO
- Spectral similar to Cy 5.5, IRD 700 and A680
- Negatively charged –2*, and highly water soluble
- Enhanced water solubility and polarity

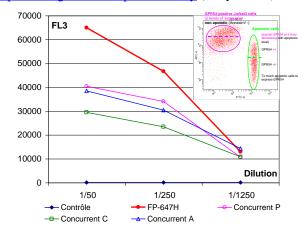


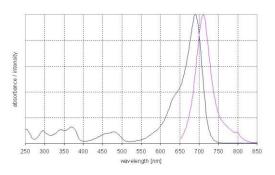


As a result, FluoProbes®647H is:

- a superior alternative to TMR fluorophores, Cy5 and A647.
- It suits **any fluorescent techniques**: microscopy fluorescence, microplate and microarray assays, FCM...

Superior brightness and photostability (to Cy5, A647)





Absorption and emission spectra

More information

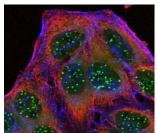
*Also available as a derivative with a single negative charge <u>FluoProbes681</u> and un-charged <u>FluoProbes680</u>.

FluoProbes®

 $FT\text{-}FPstd_(StandardSelectedFluoProbesLabels)$

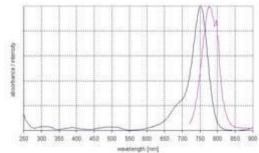
Fluoprobes®752 label:

- Very bright Infra-Red fluorescence $(\lambda_{exc}./\lambda_{em.}$: 748 / 772nm; EC: 270 000)
- Negatively charged -2, and highly water soluble
- High brightness
- Compatible with standard filters for Cy 7...



More information

*also available as a single negative charge version (<u>FP751</u>) and no charge (<u>FP750</u>).



Derivative	FluoProbes752	FP751	FP750
-COOH	FP-IS2780	FP-BA4290	FP-BA4250
$-NH_2$	FP-IS2800	FP-BA4300	FP-BA4270
-NHS	FP-BF6570	FP-AZ3520	FP-BA4260
-MAL	FP-IS3010	FP-BA4310	FP-AB4280
-Lab.kit	Inquire	FP-CG5400	

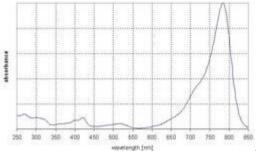
Fluoprobes®782 label:

- **bright IR fluorescence** (λ_{exc}./λ_{em.}: 738 / 800nm; EC: 170 000)
- Soluble in methanol, ethanol, DMF, DMSO
- Double negatively charged *
- Has been used combined to FP680 for 2D-DIGE

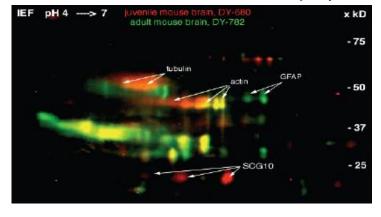
Derivative	FluoProbes®782	FP781
-COOH	FP-BA4400	
$-NH_2$	FP-BA4410	
-NHS	FP-AY6590	
-MAL	FP-BA4420	
-Lab.kit	FP-CA6070	

*also available as a single negative charge version, FP781

More information



Absorption spectrum





FT-FPstd (StandardSelectedFluoProbesLabels)

Scientific and technical Information - Derivates/coupling

Fluoprobes[®] **labels** are available as different derivatives, suiting standard chemistry methods (seek in the literature the righ conjugation strategy for your application), and others (dying materials,...):

Carboxylic derivatives are can be used for any kind of spectroscopy, and coupled to biomolecules by conventional chemistry, i.e. after activation at the carboxy group by EDC.

Carboxylic derivatives are stored at ambient temperature and is stable for at least three years.

Amine derivatives can be used for any kind of spectroscopy, and coupled to biomolecules by conventional chemistry, i.e. by reaction with aldehydes, or with carboxyls by amidation mediated with EDC.

Amine derivatives are stored at ambient temperature and is stable for at least three years.

NHS-ester derivatives are suited for direct labeling of amino groups in proteins and aminated DNA/RNA. The chemical group N-hydroxysuccinimidyl (NHS) reacts optimally at neutral pH or higher in aqueous phase on primary (– NH2) and secondary amines (-NH-) (in fact on its deprotonated form): i.e. amines present in proteins (Lys aminoacid) and in a lower proportion on NH2 located in terminal peptidic chains. The reaction competes with hydrolysis that increases with pH, and with the high dilutions of the molecule that should be labeled. Please refer to the literature, or the technical sheet FT-BA6800 (NHS-FluoProbes labels) for a standard protein coupling protocol.

NHS-esters should be stored at 0-4°C protected from moisture, and is stable for at least one year (–20°C for long term).

Maleimide derivatives are suited for labeling of thiol groups of proteins or other molecules, e.g. specific labeling of cysteine. Please refer to the literature, or the technical sheet <u>FT-BA6810</u> (Maleimide-FluoProbes labels) for a standard protein coupling protocol.

Maleimide derivatives should be stored at 0-4°C and are stable for at least one year (or RT/short term, or -20°C/long term).

Hydrazide derivatives are suited for labeling of biomolecules. Please refer to the literature, or the technical sheet <u>FT-BA38820</u> (Hydrazide-FluoProbes labels) for a standard protein coupling protocol.

Hydrazide derivatives should be stored at 0-4°C and are stable for at least one year (or RT/short term, or -20°C/long term).

You also may ask Fluoprobes for labeling kits, several already prepared conjugates such as Streptavidin, antibodies and some specific probes (i.e. AnnexinV-FP488), and custom labeling.





Other remarkable FluoProbes labels

Product name	MW £	$\lambda_{exc} \setminus \lambda_{em}$. max.	mol. abs.	Comments
cat.number	(g·mol⁻¹)	(nm)	$(M^{-1}cm^{-1})$	
FluoProbes®415	573.6	418 / 467	34 000	 A good blue dye, alternative to DAEC/AMCA More information
FluoProbes®465A	492.9	453 / 508	75 000	A good blue/vert dyeMore information
FluoProbes [®] 505	566.1	505 / 530	80 000	 Green/Yellow. A good alternative to RhodamineGreen, FITC, Cy2 More information
FluoProbes®532A	646	532 / 553	115 000	 Yellow. A good alternative to A532. Excitable at 515 - 545 nm (YAG) More information
FluoProbes®565A	708.1	563 / 553	115 000	 Orange/red. A good alternative to Bodypi568 & more photostable than Cy5. More information
FluoProbes®590A	788.3	594 / 624	120 000	Red. A good alternative to TR/SR101/A594 More information
FluoProbes®594A	1389	601 / 627	120 000	Red. A superior Alternative to TR/SR101/A594 More information
FluoProbes®633A	848.0	637 / 657	200 000	 Red. A good alternative to A633, A647 More information
FluoProbes®680A		680 / 700	125 000	Far Red/IR. Alternative to Cy TM 5.5 More information
FluoProbes® XXL				• Extra long stock's shift – up 5 dyes can be used with one light source
(long stock's shift)				• More information (FP480XXL, 481XXL, 510XXL, 520XXL, 521XXL)
And much more!				list of FluoProbes dyes NHS esters
AF dyes (more [FPAF_])				 Similar to AlexaFluor®. Functionalized by NHS (R08112), Azide (AXCJ91), PicolylAzide (AYH9B1), Alkyne (AXCECA), Maleimide (820731), Hydrazide (846631), Amine (), Carboxyl ()

£: MW of NHS ester

NT-FPcompa Compatible light sources (excitation) and filters (emission) for FluoProbes labels.

 $Fluo Probes^{\circledast} \ is \ a \ registered \ trademark \ from \ Interchim \\ Cy^{TM} \ is \ a \ registered \ trademark \ from \ Amersham \ Biosciences \ Corp. \\ AlexaFluor \ is \ a \ registered \ trademark \ from \ Molecular Probes$

Ordering information

Search all products at www.interchim.com/interchim/customers/
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask: FluoProbes® / Interchim; Hotline: +33(0)4 70 03 73 06

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Rev.IIIE-HIIE-HO3E