Advion Interchim scientific

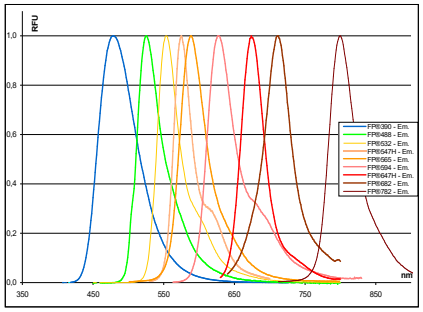
NHS-FluoProbes® labels

NHS-Fluoprobes® are great fluorescent agents for labeling proteins and any amine-containing molecules.

Products Information

Product name (NHS ester) cat.number	**MW** ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em.}$ **max.** (nm)	**mol. abs.** ($M^{-1}cm^{-1}$)	**Comments**	
NHS-FluoProbes® 390A FP-BS5620, 1mg	440.5	390 / 479	24 000	• Bright blue fluorescence, great for double and triple detections • Compatible with standard filters for AMCA	More information
NHS-FluoProbes® 419S FP-M17181, 1mg	339.2	419 / 452	24 000	• Bright ocean blue fluorescence • Compatible with blue diode (violet) laser	
NHS-FluoProbes® 488 FP-BA6800, 1mg	981	493 / 518	85 000	• Bright green fluorescence. • pH-independent fluorescence between pH 2 and 8 • Ultimate photostability, hence minimal fading • Compatible with standard filters for FITC, $CY_{anine}2$ • Ideal for confocal microscopy, but suits also any other technique, including microplate readers and FCM.	More information
NHS-FluoProbes® 488D FP-BG1HY0, 1mg	1003.19	493 / 518	73 000	• Bright green fluorescence, pH-stable emission • Soluble in water, methanol • Compatible with standard filters for FITC, $CY_{anine}2$ • Ideal for flow cytometry, FisH microscopy, gel electrophoresis	
NHS-FluoProbes® 490 FP-JO2820, 1mg	1011.2	491 / 515	73 000	• Brightest green fluorescence. • pH-independent fluorescence between pH 2 and 8 • High photostability • Ideal for confocal microscopy and flow cytometry	More information
NHS-FluoProbes® 532A FP-BA6950, 1mg	743	532 / 553	115 000	• Bright yellow fluorescence • Suit for the 532nm line of the frequency-doubled Nd:YAG laser.	More information
NHS-FluoProbes® 547H FP-1H0880, 1mg	1040.06	550 / 575	150 000	• Bright orange fluorescence • Compatible with standard filters for $CY_{anine}3$, Rhodamine TRITC • High brightness • Improved water solubility	More information
NHS-FluoProbes® 565A FP-BA7040, 1mg	708.1	563 / 592	120 000	• Bright orange fluorescence	More information
NHS-FluoProbes® 594A FP-BU7160, 1mg	1389	603 / 626	120 000	• Bright red fluorescence	
NHS-FluoProbes®647H FP-1H0930, 1mg	1066.10	655 / 676	250 000	• Bright red fluorescence • Compatible with standard filters for $CY_{anine}5$ • High brightness • Improved water solubility	More information
NHS-FluoProbes® 682 FP-BE6200, 1mg	950.03	690 / 709	140 000	• Infra Red fluorescence • Compatible with standard filters for $CY_{anine}5.5$, IRD700™ • High brightness • Improved water solubility	More information
NHS-FluoProbes® 782 FP-AY6590, 1mg	976.07	783 / 800	170 000	• Infra Red fluorescence • Compatible with standard filters for IRD800™ • High brightness • Double negatively charged	More information

Storage: NHS ester derivatives should be stored at +4°C [or –20°C (long term)] (K')



FluoProbes® labels series

Fluoprobes® provides a full range of fluorophores to covers any applications, spanning from 390nm to 800nm. **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, as well as dying materials.
Please see selected most popular and remarkable FluoProbes labels used in standard applications (i.e. blue, green, orange, red, infrared).
Please contact FluoProbes if you have original applications and great images. You may be eligible for a reward!

Introduction

Fluoprobes®-NHS esters are great labeling agents for amine containing molecules (proteins, amino-allyl nucleotides,...). Main fluorescent features are displayed in above table. See the technical notice 'FT-FPxxx_' for technical information about fluorescence characteristics of the FluoProbes® labels.

The chemical group **N-hydroxysuccinimydyl** (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. More information on inquire.

Directions for Use

Here are standard protocols for proteins (1) and aminated nucleotides (2). The labeling conditions may be adapted or optimized depending on quantity, volume or concentration, desired labeling degree, susceptibility to inactivation (i.e. ceratin antibodies, especially monoclonal): ratio of NHS-FluoProbes® / molecule, temperature, duration of incubation, procedure of purification.

Protocol 1 : antibody labeling

This simple and quick standard protocol labels polyclonal and monoclonal purified antibodies. It suits also most proteins and peptides using a similar ratio of NHS-FluoProbes® / protein or peptide concentrations.

1– Prepare the antibody at 5mg/ml in PBS (NaCl 150mM, Phosphate 20mM, pH7.5).

Other pH7-9 buffers could be used provided they do not contain amines. Lyophilized Ab can be dissolved directly with PBS buffer, concentrated Ab can be diluted. The protocol will work down 2mg ab /ml concentration, i.e. in sodium carbonate buffer pH 8.3, but for lower concentrations, the NHS/protein coupling ratio should be slightly increased.

2– Prepare a NHS-FluoProbes® solution at 10mM in anhydrous DMSO.

Note: NHS-FluoProbes® can be dissolved in other organic solvents as dimethylformamide (DMF). FluoProbes® recommends not to store the stock solution, because the product is readily subject to hydrolysis. A limited storage may be possible when using high quality anhydrous solvents under argon or nitrogen gas at –20°C.

3– Add 40-80µL of the solution of NHS-FluoProbes® to the antibody (1ml). Incubate 1H at room temperature.

Note 1 : the possible conditions of the esterification reaction are various. The labeling is usually realized in a neutral buffer, like PBS (NaCl 150mM, phosphate 20mM, pH7.4), or carbonate (but not in Tris buffers).

Note 2 : the protein solution should be in 2 to 4 fold molar excess over the activated dye (2 mg/ml in e.g. acetonitrile).

4– Desalt the antibody by dialyzing against PBS+NaN_3 0.01% (i.e. with CelluSep membranes). The labeled antibody can be diluted to 1mg/ml with 0.1% NaN3 and 20% of glycerol for storage at–20°C (long term) or +4°C.

Note: other suitable techniques may be used, as gel filtration (e.g. using desalting column G-25, # 84874, or other toolsr). For some applications it may be not necessary to remove after labeling the by-products (free FluoProbes® dye and NHS resulting from conjugation and hydrolysis of excess of NHS-FluoProbes®).

Determining the degree of labeling

The level of labeling is in the range of 1-5 dyes per IgG, but depends on the efficiency of a labeling reaction and protein type. the approximate number of dye molecules conjugated per protein molecule can be determined, using the Beer-Lambert law, from the absorbance of the conjugate at 280 nm (for protein) and the absorbance of the dye at its maximum absorbance wavelength (λmax). The λ_{max} value and the extinction coefficient (ε) of the dye are given in the table at the beginning of this product information sheet.

• Measure the absorbance of the protein–dye conjugate at 280 nm ($A_{280[protein]}$) and at the dye λ_{max} ($A_{max[protein]}$):
Dilute the protein–dye conjugate to approximately 0.1 mg/mL, so you have a 0.5-3 OD absorbance.

FT-BA6800

• Determine the concentration of the protein from $A_{280[protein]}$ corrected for the contribution of the dye at 280nm (correction factor CF) and assuming 1.4 Abs units = 1 mg protein/mL (this value is correct for IgG antibodies, but may be different for your protein).

[protein] = 1.4 x ($A_{280[protein]}$ - $A_{max[protein]}$ x CF)

$$CF = \frac{A_{280\ free\ dye}}{A_{max\ free\ dye}}$$

Calculate the Dye / Protein ratio (degree of labeling D.O.L.):

where MW = molecular weight of the protein

ε_{dye} = the extinction coefficient of the dye at its absorbance maximum in $cm^{-1}M^{-1}$

[protein]= protein concentration in mg/mL.

$$DOL = \frac{A_{max} \times MW}{[protein] \times \epsilon_{dye}}$$

The optimization of the labeling degree is classically performed by conjugating the antibody with several ratios of NHS-FluoProbes® / protein, below and above the standard ratio, then by testing the labeled antibodies directly in the application to select the best result.

Protocol 2 : Labeling aminoallyl nucleic acids (& incorporation of aa-dUTP by Reverse Transcription)

AminoAllyl-UTP (aa-UTP) is incorporated for subsequent labeling by NHS-FluoProbes®dye.

1– *Make the dNTP + aa-dUTP mixture:*

	Volume (µL)
100mM dATP	10
100mM dCTP	10
100mM dGTP	10
100mM dTTP	6
100mM aa-dUTP	4
total dNTP + aa-dUTP mixture (50x)	*40µL*

2– Set up the Priming Reaction:

	[concentration]	*µL*
Oligo dT / Random Primer	2µg/µL each	1
poly(A)+ RNA	2µg total	14.5
total Priming Reaction per reaction		*15.5µL*

3– Incubate the priming reaction at 70°C for 8 minutes. Remove and put on ice.

4– *Set up the cDNA synthesis reaction:*

	[concentration]	*µL*
RT Buffer	10x	3
aa-dNTP	50x	0.6
DTT	0.1M	3
Reverse Transcriptase	50U/µL	2
Water		5.9
total cDNA Synthesis Reaction per reaction		*14.5 µL*

5- Add 14.5µL of master mix to each Priming Reaction.

6- Incubate reactions at 42°c for 2 hours.

Hydrolysis and Cleanup

1– Bring cDNA synthesis reactions to a final concentration of 100mM NaOH and 10mM EDTA. Incubate at 65°C for 10 minutes.

2– Neutralize the hydrolysis reaction by the addition of HEPES, pH 7.0, to a final concentration of 500mM. Other non-primary amine containing buffers may also be used. Note that Tris buffer carries a free amine and should be avoided since this could possibly interfere with the subsequent coupling reaction.

3– Bring the reaction volume to 500µL with water. Concentrate the cDNA product by filtering through an UptiSpin™ microcentrifuge filter or other similar UF device r. Try to get the final volume of the sample down to below 10µL. This can usually be accomplished by spinning at full speed typically for 6-10 minutes. Do not spin to dryness as this can make the cDNA difficult to recover.

4– Bring the concentrated product to 500µL and repeat the concentration at least twice. The net effect of this process is to remove the hydrolyzed RNA, NaOH, and buffer components.

5– The amino-allyl labeled cDNA may now be stored indefinitely at -20C.

Aliquoting FluoProbes®-dye esters

1. Resuspend the solid pellet in 12µL of water free DMF or DMSO.

2. Since a single tube of dye usually provides sufficient material to label at least 12 samples, aliquot 1µL volumes of the resuspended dye into separate screw cap tubes. Dry down the aliquots using a speed-vac, without heat.

3. Store the dye aliquots at 4C, in a light-sealed box, preferably under vacuum and in the presence of a large amount of desiccant; This will help ensure the dyes remain uncontaminated with moisture.

Coupling to N-hydroxysuccinimidyl ester dyes

1– Bring the cDNA solution to a final volume of 10µL with water. Adjust pH to 7-8 with 1M sodium bicarbonate buffer pH 9.0.

2– Remove a dye aliquot from storage and use the bicarbonate buffered cDNA solution to vigorously resuspend the pellet by pipetting up and down.

3– Incubate the coupling reaction in the dark for at least 60 minutes at room temperature.

Removal of uncoupled dye material

Use PCR Purification columns to remove uncoupled dye.

Concentrate the eluate to desired volume by vacuum drying or by concentration using a UptiSpin filter.

References

- **Brumbaugh J. A**., *et al.*, "Continuous, online DNA sequencing using oligodeoxynucleotide primers with multiple fluorophores.", *Proc Natl Acad Sci U S A* **85**(15)**,** 5610-4(1988) (Amino allyl Coupling)
- **Hughes T. R**., et al., « Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer.", *Nat Biotechnol* **19**(4)**,** 342-7(2001) (Amino allyl Coupling)
- **Montet-Abou K**. *et al*., *In vivo* labelling of resting monocytes in the reticuloendothelial system with fluorescent iron oxide nanoparticles prior to injury reveals that they are mobilized to infarcted myocardium, *European Heart Journal*, Vol. 31, Issue 11:1410-1420 (2010) Abstract (FP565)
- **Randolph J. B**., *et al.,* "Stability, specificity and fluorescence brightness of multiply-labeled fluorescent DNA probes.", *Nucleic Acids Res* **25**(14)**,** 2923-9 (1997) (Amino allyl Coupling)
- **Roth L**. *et al*., Transmembrane Domain Interactions Control Biological Functions of Neuropilin-1, *MBoC*, Vol. 19, Issue 2, 646-654 (2008) Article

Related products

- Buffers
- Desalting: UptiSpin filters; Gelfiltration G-25 columns # 84874; CelluSep dialysis tubings
- Aminoally-dUTP, FP-AK218A
- PCR Purification columns, AP3870
- FluoProbes® Protein labeling Kits (FP488 FP-BE3750)
- Secondary Abs: i.e. for multiple color detections:
- FluoProbes® 547H Goat anti-Mouse IgG, FP-SB4000
- FluoProbes® 547H Goat anti-Rabbit IgG, FP-SB5000
- FluoProbes® 647H Goat anti-Mouse IgG, FP-SC4000
- FluoProbes® 647H Goat anti-Rabbit IgG, FP-SC5000

More FluoProbes labeling agents

See selected most popular and remarkable labels and the list of FluoProbes-NHS derivatives.
Other derivatives are available, incl. amino-, carboxy-, Maleimide-, Hydrazide-, (strept)avidin, secondary antibodies, some specific probes such as Annexin, Phalloidin, ... or any other on custom labeling.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please 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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