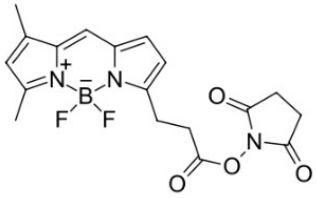
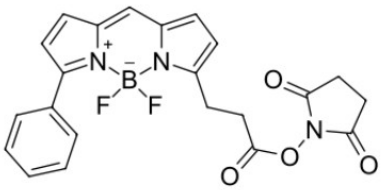
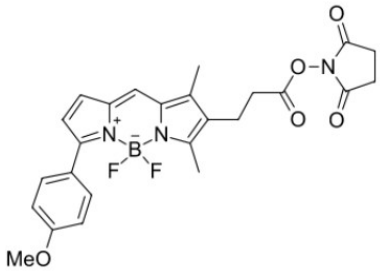
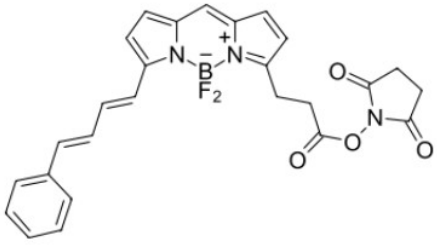
FluoProbes®

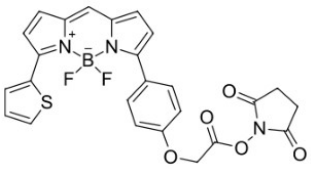
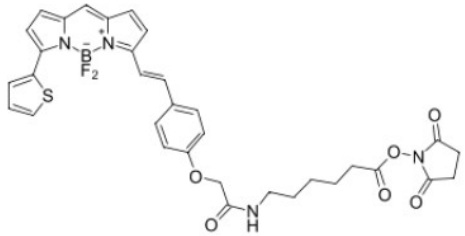
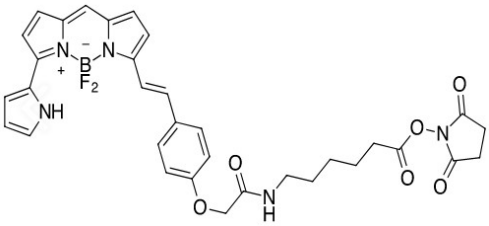
FT-23383A

BrDIPY NHS ester

Amine-Reactive Neutral Fluorescent probe for synthesis of green-fluorescent biomolecules

Products Description

Product name cat.number/qty*	**MW** ($g \cdot mol^{-1}$)	**λ abs./em.** (nm)	**mol. abs.** ($M^{-1}cm^{-1}$)	Comment, structure
BrDIPY FL–NHS ester (M) FP-233831, 1mg FP-233832, 5mg FP-233833, 25mg FP-233834, 50mg FP-233836, 100mg	389.16	503 / 509	82000 QY: 0.97	 4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Propionic Acid, Succinimidyl Ester CAS [146616-66-2]
BrDIPY R6G–NHS ester (M) FP-379931, 1mg FP-379932, 5mg FP-379933, 25mg FP-379934, 50mg FP-379935, 100mg	437.21	530 / 548	70000 QY: 0.96	 2,5-Dioxo-1-pyrrolidinyl 3-(4,4-difluoro-5-phenyl-3a,4a-diaza-4-bora-s-indacen-3-yl)propionate CAS [335193-70-9], [1443457-59-7]
BrDIPY TMR–NHS ester (M) FP-0C3070, 1mg FP-0C3071, 5mg FP-0C3072, 25mg FP-0C3073, 50mg FP-0C3074, 100mg	495.28	545 / 570	60000 QY: 0.95	 CAS [485397-12-4]
BrDIPY 581/591–NHS ester (M) FP-M12001, 1mg FP-M12002, 5mg FP-M12003, 25mg FP-M12004, 50mg FP-M12005, 100mg	489.28	585 / 594	104000 QY : 0.83	

Product name cat.number/qty*	MW (g·mol^{-1})	λ abs./em. (nm)	mol. abs. (M^{-1}cm^{-1})	Comment, structure
BrDIPY TR–NHS ester FP-AWHFN0, 1mg FP-AWHFN3, 5mg FP-AWHFN2, 25mg FP-AWHFN4, 50mg FP-AWHFN1, 100mg	521.30	589 / 616	69000 QY : 0.9	 Methyl {p-[4,4-difluoro-5-(2-thienyl)-3a,4a-diaza-4-bora-s-indacen-3-yl]phenoxy}acetate CAS [150152-65-1]
BrDIPY 630/650–NHS ester FP-AWHFC0, 1mg FP-AWHFC3, 5mg FP-AWHFC2, 25mg FP-AWHFC4, 50mg FP-AWHFC1, 100mg	660.5	628 / 642	97000 QY: 0.91	 Succinimidyl-6-[2-(p-{(E)-2-[4,4-difluoro-5-(2-thienyl)-3a,4a-diaza-4-bora-s-indacen-3-yl]ethenyl}phenoxy)acetylamino]hexanoate CAS [2213445-35-1] [380367-48-6]
BrDIPY 650/665–NHS ester FP-584631, 1mg FP-584632, 5mg FP-584633, 25mg FP-584634, 50mg FP-584636, 100mg	643.45	649 / 667	94000 QY: 0.52	 CAS [235439-04-0] [1616842-78-4]

*** Storage:**

***BrDIPY NHS ester derivates*:** –20°C for long term, protected from light (M, L)

*** Technical information about the dyes :**

BrDIPY dyes are useful fluorophores for developing fluorescent intracellular tracers. Its conjugates are cell-permeable as long as its carriers are cell-permeable.

*** Technical information about the 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.

- 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.

- 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.

- 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 tools). 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.
- 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)

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

3– 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.

<u>Protocol 2</u> – 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– <u>Make the dNTP + aa-dUTP mixture:</u>

	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.

Related products

- FluoProbes FL C5, Neutral probe, FP-M1270A
- FluoProbes FL EDA, Neutral probe, FP-M1203A
- FluoProbes FL Hydrazide, Neutral probe, FP-85357A
- FluoProbes FL propionic acid, Neutral probe, FP-19749A
- FluoProbes FL Verapamil, Neutral Probe, FP-39736A
- FluoProbes TMR C5-maleimide, Neutral probe, FP-MB171A
- FluoProbes TMR-X, SE, Neutral probe, FP-M1305A
- FluoProbes TR-X, SE, Neutral probe, FP-M1304A

Ordering information

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