

FluoProbes® 521XXL labeling agents

Product Information

An extra-long Stokes shift fluorophore for labeling biomolecules with fluorescent *far red* emission

cat.number	MW (g·mol ⁻¹) M+	$\lambda_{exc}/\lambda_{em}$ max. (nm)	mol. abs. (M ⁻¹ cm ⁻¹)	Quantum yield (%)
Fluoprobes® 521XXL - Carboxyl group (L) FP-BA6280, 1mg	514.6	523 / 668	50 000	
Fluoprobes® 521XXL - Amino (K) FP-BA6300, 1mg	593.1	<ul style="list-style-type: none"> Excitable as fluoresceins are extra-long Stokes shift Red fluorescence Soluble in Water 		
Fluoprobes® 521XXL - NHS (K) FP-BA6290, 1mg	611.7			
Fluoprobes® 521XXL - Maleimide (M) FP-BA6310, 1mg	636.7			
Fluoprobes® 521XXL - STP ester () FP-C03310, 1mg	764.7			
Fluoprobes® 521XXL - Labeling Kit (L) Inquire, 1Kit (5runs)	-			
Other Fluoprobes® 521XXL products See related products				

Storage: (L): at +4°C (K): at +4°C long term at -20°C (M): at -20°C

Scientific and technical Information - Label

Fluoprobes® 521XXL is part of the **Fluoprobes® dyes series**.

Fluoprobes® provide 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 dyeing materials.

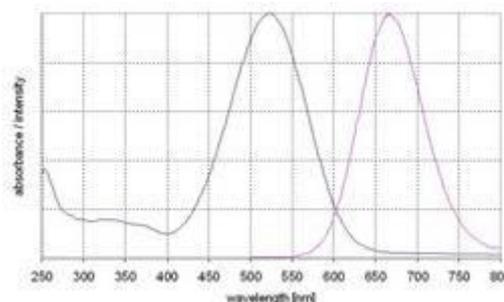
Fluoprobes® XXL dyes have to particularity to have long to extraordinarily long Stokes's shift. FP480XXL, 481XXL, 485XXL, 510XXL, 520XXL and 521XXL can be used with a single light source suitable to fluoresceins, while emitting in increasing wavelengths. They are available combined in a starter set FP-BA2021, each with NHS reactivity.

Fluoprobes® 521XXL Label features:

- can be excited by the 488 line of Argon laser.
- far red fluorescence** ($\lambda_{exc}/\lambda_{em}$: 523/668nm in EtOH):
(ϵ at λ_{max} : 50 000 M⁻¹cm⁻¹).
- Soluble** in Water, MeOH, DMF, DMSO

FluoProbes®521XXL is:

- ideal for use combined with Fluorescein with single source excitation**
- suits protein labeling for microarray, FISH microscopy, gel electrophoresis



Absorption and emission spectra

Scientific and technical Information - derivatives

Fluoprobes® 521XXL is available as different derivatives, suiting standard chemistry methods.

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

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 by carbodiimides (EDAC).

Please refer to the literature for protocols, or the technical sheet [FT-52005A](#) (EDAC):

Storage : Carboxylic acid and mine derivatives are stored at ambient temperature and is stable for at least three years.^(L)

NHS-ester derivatives are suited for direct labeling of amino groups in proteins and aminated DNA/RNA.

The chemical group N-hydroxysuccinimidyl (NHS) reacts specifically with primary (-NH₂) and secondary amines (-NH-) (in fact on its deprotonated form) in aqueous phase or at pH 8 (compatible with pH7 to 10) in PBS buffer (other buffer devoid of amines are possible) at a ratio of 1-6 over amine content. 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 for protocols, or the technical sheet [FT-BA680](#) (NHS-FluoProbes labels):

Storage : NHS-esters can be stored at 0-4°C, stable for several months. They should be protected from moisture and light.^(K)

Maleimide derivatives are suited for labeling of thiol groups, e.g. specific labeling of cysteine of proteins or peptides.

The maleimide group reacts very specifically with sulfhydryls -SH at neutral pH 6.5-7. The reaction is rapid (a few minutes for cysteine), but in the absence of -SH, maleimide stay well stable. In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein.

Please refer to the literature for protocols, or the technical sheet [FT-BA681](#) (Maleimide-FluoProbes labels):

Storage : Maleimide derivatives can be stored at 0-4°C, stable for several months, or at -20°C for long term. They should be protected from moisture and light.^(M)

Hydrazide derivatives give a variety of reactions:

.react specifically with **aldehydes and ketones**, forming an hydrazine bond.

.react with **carboxyls**, that better occur with activation by a carbodiimide (EDC).

.react also with **amines** forming an intermediate Schiff's base that can be further stabilized by reduction .

.other reactions are possible as well (with cytosine, NHS)

Hydrazide provides thus privileged methods to conjugate a variety of biomolecules: glycoproteins, glycolipids, sialic acids and sugars, steroids, LDL and nucleic acids, but also N-terminal serine and threonine residues in proteins. For reducing sugars (containing free CHO groups), direct conjugation is possible, but most other applications require a reducing or an oxidising step to generate CHO groups from carboxyls or from cis-diols.

Please refer to the literature for protocols, or the technical sheet [FT-CE0640](#) (Hydrazide-FluoProbes labels):

Storage : Hydrazide derivatives should be stored at 0-4°C and are stable for at least one year.

Azide derivatives yield useful reactions:

.they are also popular for their participation in bioorthogonal reactions,

the "Click Chemistry" (with to alkyne-modified molecules, forming a stable triazole bond), and

the Staudinger ligation (with Phosphine-modified molecules, forming an amide bond).

.they are commonly used as a way to introduce an amine group,

.they participate to other kind of reactions as well, for example azide react with carbonyls or ketones to form amine or amides (Schmidt reaction); and arylazides react with amines

Please refer to the literature for protocols, or the technical sheet [FT-YE4970](#) (Azide-FluoProbes labels) and [FT-ZL5530](#) (Click Chemistry reagents):

Storage : Azide derivatives should be stored at 0-4°C and are stable for at least one year. ^(L)

Alkyne derivatives provide a nice partner for Click Chemistry based conjugations with Azides: the Alkyne-FluoProbes can be click-conjugated to azide-modified molecules, through a stable triazole bond.

Please refer to the literature for protocols, and above information for Azide protocols.

Storage : Alkyne derivatives should be stored at 0-4°C and are stable for at least one year. ^(L)

General advice

In any bioconjugation, a calibration of dye/biomolecule ratio may be needed to optimize the labeling level depending on molecule and application, i.e. adjust concentration weight of the FluoProbes® dye / weight of protein or peptide. Then the parameters of the detection instrument should also be set properly for FluoProbes dye (see above/label).

Info@fluoprobes.com

Technical-support@fluoprobes.com

Order-online@fluoprobes.com

Contact your local distributor

FluoProbes®, powered by



213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

FT-FP521X

Related products

FP-IS1790: Streptavidin-FP521XXL FP-IS2540: Avidin-FP521XXL FP-IS3530: Biotin-FP521XXL

[All FluoProbesXXL long-stock's shift dyes](#) (FP480XXL, 481XXL, 510XXL, 520XXL, 521XXL / activated by Carboxyl, Amine, NHS, Maleimide, Hydrazide, Azide, Lab.Kit)

[Selection of the most remarkable and used FluoProbes](#) ^[FT-FPstd_]

for standard applications i.e. blue, green, orange, red, infrared).

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

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213 Avenue J.F. Kennedy - BP 1140
03103 Montluçon Cedex - France
Tél. 04 70 03 88 55 - Fax 04 70 03 82 60