

FluoProbes® 547

To prepare the brightest fluorescent conjugates with *orange* emission

Product Information

| cat.number | MW (g·mol ⁻¹) | $\lambda_{exc}/\lambda_{em}$ max. (nm) | mol. abs. (M ⁻¹ cm ⁻¹) |
|--|---|--|--|
| Fluoprobes® 547 - Carboxyl group (L) FP-1J3680, 1mg | 682.79 | 551 / 565 (in PBS) 559/575nm (in ethanol) | 150 000 |
| Fluoprobes® 547 - Amino group (L) FP-1J3690, 1mg | 702.90 | | |
| Fluoprobes® 547 – NHS (K') FP-1J3700, 1mg | 779.87 | | |
| Fluoprobes® 547 – Maleimide (M) FP-1J3710, 1mg | 804.92 | | |
| Fluoprobes® 547 – Azide (M) FP-1J3720 | | | |
| Fluoprobes® 547 Labeling Kit (V) FP-BC0900, 1kit (5 runs/1.5mg prot) | - | | |
| other Fluoprobes® 547 products | Please related product , or consult the BioSciences catalogue | | |

Storage: (L): at +4°C (K): at +4°C (long term at -20°C) (M): at -20°C
(V): dye at 4°C or -20°C and other components at room temperature

FluoProbes®547 is our original popular fluorochrome with orange emission. We recommend now for labeling applications the more hydrophilic version FluoProbes547H, that can achieve even higher coupling ratio without quenching notably for proteins.

Scientific and technical Information - Label

Fluoprobes® 547 label offer great advantages:

- **Bright orange fluorescence**

$\lambda_{exc}/\lambda_{em}(\text{ethanol})$: 557/574 nm

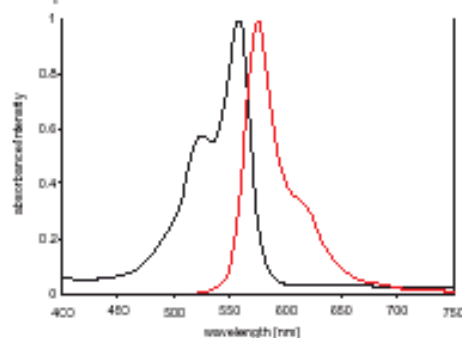
FP®547H shows elevated extinction coefficient and can usually be coupled at high ratios without quenching. FP®547H has higher signal and lower background than other standard orange fluorophores (TMR, AF546), and reduce the fading observed in some applications with Rhodamine TRITC, Cy3 labels.

- pH-independent fluorescence
- Compatible with standard filters for TRITC/CyTM3...

As a result, FluoProbes® 547H is:

- **a superior alternative to TMR fluorophores, CyTM3 AF555 and AF546** (see [comparison](#))
- It suits **any fluorescent techniques**: microscopy fluorescence (including confocal microscopy), microplate and microarray assays, FCM...

Absorption/Emission of FluoProbes®-547



*note: FluoProbes 547 label is available as a more hydrophilic and polar version, [FluoProbes 547H](#), that is recommended for some applications, i.e. labeling at high coupling ratios.

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Contact your local distributor FluoProbes®, powered by [interbiotech@interchim.com](#)



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Scientific and technical Information - Derivate

Fluoprobes® 547H is available as

- different derivatives (see above) suiting labeling with standard chemistry methods
- already coupled to several specific ligands (see related products).
- the more hydrophilic and polar version [FP547H](#) (great for labeling, especially for proteins).

Storage and General uses

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 EDC.

Carboxylic derivatives are stored at ambient temperature and are 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-hydroxysuccinimide (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, or the technical sheet [FT-BA6800](#) (NHS-FluoProbes labels) for standard protocols.

NHS-esters 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.

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

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, or the technical sheet [FT-BA6810](#) (Maleimide-FluoProbes labels) for standard protocols.

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.

Hydrazide derivatives are suited for labeling of biomolecules. Please refer to the literature, or the technical sheet FT-B3882 (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 (at room temperature for short term, or at –20°C for long term).

You also may ask **Protein labeling kits**, already prepared **Fluoprobes conjugates** (see related products), and **custom labeling**.

Standard protocols (*)

Protocol 1 : antibody labeling with NHS ester

See the technical sheet [FT-BA6800](#). This simple and quick standard protocol labels polyclonal and monoclonal purified antibodies for immunodetection applications. It suits also most proteins and peptides (*).

Protocol 2 : Incorporation of aa-dUTP by Reverse Transcription

See the technical sheet [FT-BA6800](#). AminoAllyl-UTP (aa-UTP) is incorporated in nucleic acids using a DNA polymerase (PCR, Nick translation) for subsequent labeling by NHS-FluoProbes® dye.

Protocol 3 – protein labeling with maleimide

See the technical sheet [FT-BA6810](#). Fluoprobes® dye maleimide is suited for labeling of proteins at cysteine sites.

(*)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).

Other FluoProbes® 547 labeled products:

| Conjugates of | FluoProbes® 547 | FP547H conjugates | FP547H lab.agents |
|---|-----------------|--|------------------------------------|
| Fluoprobes® 547 - Streptavidin | FP-AX1460, 1mg | FP-CA5570, 1mg | FP547H-COOH: FP-1H0880 |
| Fluoprobes® 547 - Avidin | FP-BA6430, 1mg | | FP547H-NH ₂ : FP-1H0900 |
| Fluoprobes® 547 - Phalloidin | FP-AZ0330, 1mg | FP-BZ9620, 1mg | FP547H-NHS: FP-1H0890 |
| Fluoprobes® 547 - Goat Anti Mouse IgG(H+L) | FP-BZ0750, 1mg | FP-CB1020, 1mg | FP547H-MAL: FP-1H0900 |
| Fluoprobes® 547 - Goat Anti Rabbit IgG(H+L) | FP-BZ0760, 1mg | FP-CB1050, 1mg | FP547H lab.Kit: FP-BZ9600 |
| Fluoprobes® 547 – Anti Fluorescein | FP-BT3560 | *FP547 is the original version (less hydrophilic)* | |
| Fluoprobes® 547 – BSA | FP-BC2170 | FP647-Biotin | FP-IS3330 |
| Random Nanomer Oligo FluoProbes 547 | FP-BU8750 | Custom labeling | Inquire |

List of [updated list of FluoProbes dyes NHS esters](#) at <http://www.interchim.fr/ft/F/FPlistN.pdf>.

Contact your local distributor

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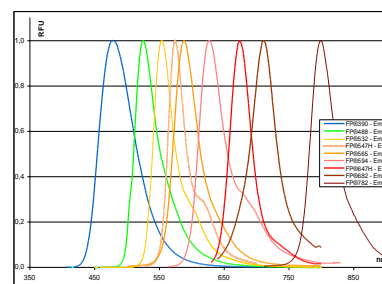


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FT-FP547'_

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.

Please see a presentation of [selected most popular and remarkable FluoProbes labels](#) in standard applications (i.e. blue, green, orange, red, infrared), or at pages [B51-B57](#) of the [BioSciences catalogue](#), and [updated list of FluoProbes dyes NHS esters](#).



Ordering information

Catalog size quantities and prices may be found at <http://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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