FT-FP547H'



FluoProbes[®] 547H

To prepare the brightest fluorescent conjugates with orange emission

Product Information

Product name	MW	Storage	$\lambda_{exc} \setminus \lambda_{em}$. max.	mol. abs.		
cat.number	$(g \cdot mol^{-1})$		(nm)	$(M^{-1}cm^{-1})$		
FluoProbes [®] 547H - Carboxyl group FP-1H0870, 1mg	884.9	L	551 / 565 (in PBS)	150 000		
FluoProbes [®] 547H - Amino group FP-1H0900, 1mg	1062.0	L				
FluoProbes [®] 547H - NHS FP-1H0880, 1mg	982.0	Н	$\begin{array}{c} H_{\rm fl} = 90 \% & \tau_{\rm fl} = 3.4 \text{ ns} \\ CF_{260} = 0.34 & CF_{280} = 0. \end{array}$	16		
FluoProbes [®] 547H - Maleimide FP-1H0890, 1mg	1007.0	М	 Soluble in water, methanol, DMF, DMSO (enhanced water solubility and polarity compared with EP547) 			
FluoProbes [®] 547H - Azide FP-1H0970			 Three negative charges Usually combined with FP647H 			
FluoProbes [®] 547H - HydrAzide FP-1H1200						
FluoProbes [®] 547H – STP ester FP-1H1210						
FluoProbes [®] 547H Labeling Kit FP-BZ9600, 1kit (5 runs/1.5mg protein)	-	V				
other FluoProbes [®] 547H products	Please <u>related product</u>					

Storage:

+

(L): at $+4^{\circ}C$ (K) : at $+4^{\circ}$ C (long term at -20° C) (M): at -20°C (V): dye at 4° C or -20° C and other components at room temperature

Scientific and technical Information - Label

FluoProbes[®] 547H label offer great advantages:

- **Bright orange fluorescence**
 - λ_{exc} ./ $\lambda_{em.}$ (ethanol): 560/575 nm

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\lambda_{exc}./\lambda_{em} (water):
                                 553/566 nm
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FP547H shows elevated extinction coefficient. FP547H has higher signal and lower background than other standard orange fluorophores (TMR, A546_), and reduces the fading observed in some applications with Rhodamine TRITC, Cy3 labels.

- pH-independent fluorescence and photostable •
- High polarity (3 negative charges*) •
- Compatible with standard filters for TRITC/Cy^T3...

As a result, FluoProbes[®] 547H is:

- a superior alternative to TMR, Cy^T3 and A546, A555
- suits any fluorescent techniques: microscopy fluorescence (including confocal microscopy), microplate and microarray assays, FCM ...
- suits multicolor detections (i.e. combined with FP488, FP647H)
- can be used in tandem with PE, with spectra similar to PE-Cy7.

500 350 400 450 550 oth Jami

FluoProbes[®] 547H is our most popular fluorochrome with orange emission.

*FP547H is a modified version of our original FP547 (1 neg charge) label, and FP548 (2 neg charges), that may be preferred in some applications.

Contact your local distributor FluoProbes®, powered by interbiotech@interchim.com 213 Avenue J.F. Kennedy - BP 1140 213 Montluçon Cedex - France Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

FT-FP547H'

Scientific and technical Information - derivates

Fluoprobes[®] 547H is available as :

-different derivatives(see above) suiting labeling with standard chemistry methods,

- -the original version <u>FP547</u> (slightly less hydrophilic, may be useful for some applications; inquire),
- -already coupled to several specific ligands (see related products).

Storage and General uses

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 are 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 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 <u>FT-BA6810</u> (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 $0-4^{\circ}$ 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 <u>FT-BA6800</u>. 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 sat properly for FluoProbes dye (see above/label).

Other FluoProbes® 547H labeled products:

Conjugates of	FluoProbes [®] 547H	FluoProbes®547*	FP547* labeling agents	
Fluoprobes [®] 547H - Streptavidin	FP-CA5570	FP-AX1460	FP547-COOH:	FP-1J3680
Fluoprobes [®] 547H - Avidin		FP-BA6430	FP547-NH ₂ :	FP-1J3690
Fluoprobes [®] 547H - Phalloidin	FP-BZ9620	FP-AZ0330	FP547-NHS	FP-1J3700
Fluoprobes® 547H - Goat Anti Mouse IgG(H+L)	FP-CB1020	FP-BZ0750	FP547-MAL	FP-1J3710
Fluoprobes® 547H - Goat Anti Rabbit IgG(H+L)	FP-CB1050	FP-BZ0760	FP547-Lab.Kit:	FP-BC0900, 5 lab
Fluoprobes® 547H – Anti Fluorescein	FP-CE9530	FP-BT3560		

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FT-FP547H' FP-1J3730 Fluoprobes® 547H - dUTPn Custom labeling Inquire List of updated list of FluoProbes dyes NHS esters.

FP547 is the original version (less hydrophilic)

References for FluoProbes547 labeled products & Applications:

**Phalloidin-FluoProbes547 - Others references

Agbulut Onnik et al; J. Biol. Chem., Vol. 282, Issue 14, 10465-10471 (2007); "Green Fluorescent Protein Impairs Actin-Myosin Interactions by Binding to the Actin-binding Site of Myosin"; Article [Phalloidin-FluoProbes547]

Gardet Agnès et al; Journal of Virology, (2006) Vol. 80, No. 8, p. 3947-3956; "Rotavirus Spike Protein VP4 Binds to and Remodels Actin Bundles of the Epithelial Brush Border into Actin Bodies; Article [Phalloidin-FluoProbes547]

Using Phalloidin-FP547 in Confocal Microscopy with epithelial cells permeabilized with saponine ; Inquire

**Secondary Antibodies -FluoProbes547 - Please inquire

Brunner Yannick et al; Molecular & Cellular Proteomics 6.6 1007-1017; "Proteomics Analysis of Insulin Secretory Granules", Article [FP546nm anti-rabbit, FP546nm anti-mouse, FP488nm anti-rabbit, FP488nm anti-mouse, FP488nm anti-pig used in LSCM LSM 510 Meta, Zeissl

Using FP546 labeled secondary Ab in Confocal Microscopy to detect the envelop virus protein H7HA in simian cells COS7; in multiplex with A488, using Zeiss Axiovert 100M microscope LSM510 - J Virol 2004 ; Inquire

Comparisons

NT-FP547c Using Phalloiding-FP547 in Confocal Microscopy -application in 3-color experiment.

myc-tagged GRP54 receptors are detected using FP547H (secondary Abs/anti myc Ab) in HEK cells, multiplexed to Phalloiding-FP505 staining of the cytoskeletton druing apoptosis and to FluoProbes®547-H Hoechst33342 staining ot nuclei.

HNT-I09 Anticorps Secondaires FluoProbes -Superior fluorescence intensity -Superior photostability







Did you got great results with FluoProbes dyes?

Please let us know, this may be useful for other researchers, and you may be elligible for a reward!

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 dying 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 $\underline{B51}$ - $\underline{B57}$ 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).

Contact your local distributor FluoProbes®, powered by interbiotech@interchim.com 213 Avenue J.F. Kennedy - BP 1140 03103 Montluçon Cedex - France Tél. 04 70 03 88 55 - Fax 04 70 03 82 60



FT-FP547H' For any information, please ask : FluoProbes[®] / Interchim; Hotline : +33(0)4 70 03 73 06

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