Advion Interchim scientific

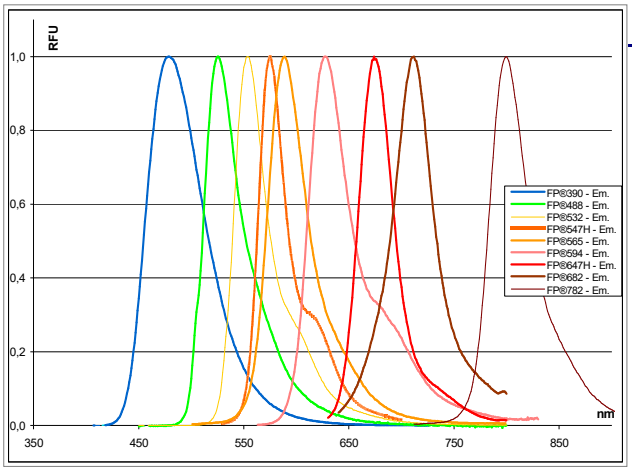
Hydrazide FluoProbes® labels

Hydrazide-Fluoprobes® are great fluorescent agents for labeling aldehyde-containing molecules, especially usefull for labeling antibodies via their glycone for improved activity

Product Information

Hydrazide Product cat.number	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ max. (nm)	mol. abs. ($M^{-1}cm^{-1}$)	Comment
Fluoprobes® 405 Blue - HYD FP-67658A, 10 mg	596.46	398 / 421	30 000	• Fluorescent cell-impermeant, fixable, polar tracer • CAS [137182-38-8] as Cascade hydrazide
Fluoprobes® 415 - HYD FP-BI1600, 1 mg FP-BI1601, 5 mg		418/467	34 000	• Suitable to substitute DEAC
Fluoprobes® 490 - HYD FP-7A3520, 1 mg		491/515	73 000	• 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 & FCM. • Soluble in water, methanol
FluoProbes® 505X5 -HYD FP-BW2860, 1 mg		505 / 530	80 000	• Highly stable, bright signal intensity • Not recommended in double labeling with orange dyes, but great with dark red or higher emission wavelengths.
Fluoprobes® 594 - HYD FP-DX2220, 1 mg	973.07	593 / 618	120 000	• Bright dark red fluorescence • Compatible with standard filters for sulforhodamine 101
Rhodamine B - HYD FP-AM531A, 100 mg	456.60	510 / 578	106 000	• Suitable for LIF detection • Probe for Cu^{2+}, NO, H_2O_2, peroxynitrite, glucose, diacetyl, hemoglobin
Fluoprobes® 647H -HYD FP-FQ6560, 1mg FP-1N1760, 1mg	- 961.08	655 676 652 / 673	250 000 250 000	• Bright red fluorescence • Compatible with standard filters for $CY_{anine}5$ • High brightness • Improved water solubility
Fluoprobes® 682 - HYD FP-CE0640, 1mg	845.01	690 / 709	140 000	• Infra Red fluorescence • Compatible with standard filters for $CY_{anine}5.5$, IRD700™ • High brightness • Improved water solubility
Fluoprobes® 782 - HYD FP-FJ7790, 1mg	893.03	783 / 800	170 000	• Soluble in methanol, ethanol, DMF, DMSO • Double negatively charged
Fluoprobes® 800 -HYD FP-1Q9230, 1mg	945.13	777 / 791	280 000	• Soluble in water, methanol, DMF • Hydrophilic

Storage: Hydrazide derivatives should be stored at +4°C (M')



Other FluoProbes® labels and conjugates

See related products

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 refer to page B51-B57 of the BioSciences catalogue and e-search tool for a complete list and technical sheet
Please see the 'FT-FPstd_' for a selection of the most remarkable and used FluoProbes labels in standard applications (i.e. blue, green, orange, red, infrared).

Introduction

Fluoprobes® hydrazide conjugates suit labeling of aldehydes, and (upon EDC mediated activation) to carboxyls . It provides thus a privilegied method 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. See below 'Coupling carbohydrates or glycoproteins'. Lastly, hydrazide allows for useful conjugation of peptides/proteins through their carboxyl groups in specific applications (oriented conjugations).

Directions for use

Handling and Storage

Fluoprobes® - hydrazide is supplied as dry powder and is stable for at least one year. It is soluble in DMSO

Coupling carbohydrates or glycoproteins

– Aldehyde groups have first to be generated if not already present on the molecule to conjugate (as in reducing oses). Sialic acids is easily oxidized with 1 mM sodium periodate (NaIO4). Other sugar groups can be oxidized effectively with 5-10 mM sodium periodate. For glycoproteins, oxidation of sugar moieties generates aldehyde groups. More conveniently, SFB reagent allow to graft easily an aldehyde on aminated molecules (i.e.proteins, nucleic acids) through an NHS acylation reaction.
– The hydrazide group reacts specifically with aldehyde and ketone groups, forming a stable hydrazone bond in a single step.

$$\text{R-CHO + Hydrazide-FluoProbes} \rightarrow \text{R-CH=N-NH-CO-(CH2)}_4\text{-FluoProbes}$$

– Compared with conventional labeling through amines (ubiquitous in proteins), the attachment through aldehydes (present on or generated on carbohydrates) is a useful approach for glycoproteins such as antibodies, and glycolipids. Conjugation via sugar moieties of antibodies typically provides the best orientation for the fluorescent label, as the sugar groups are associated with the Fc region of the antibody, while leaving the antibody active sites and light chains free to bind their target (better ab reactivity). The method however require cis diols of the sugars first be oxidized to aldehyde groups, which can then react with hydrazide-FluoProbes. In few cases this can impair the stability or reactivity of very fragile antibodies (notably monoclonals). Furthermore, monoclonal antibodies may be deficient in glycosylation. All that makes useful to validate the method also for any application, and including with other protein types.

Coupling carboxyls

– Hydrazide reacts with carboxyl groups in the presence of EDAC (#UP52005A):

FT-CE0640

R-COOH + EDAC + Hydrazide-FluoProbes → R-CO-NH-NH-CO-(CH2)4- FluoProbes

This occurs with aspartate and glutamate residues or on the carboxy terminus of proteins, ans carboxy group of reducing end of polysaccharides (oxidize sugar groups using either a specific oxidase (i.e. galactose oxidase), or 1-10 mM sodium meta-periodate (NaIO4). Oxidation with periodate is most efficient in acidic conditions (i.e. 0.1 M sodium acetate, pH 5.5), although neutral buffers such as phosphatebuffered saline can be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration into neutral buffer may be necessary to obtain optimal hydrazide reaction.

EDC reaction with COOH is usually performed in an acidic buffer (pH 4.7-5.5, but coupling can actually be accomplished in a buffer system up to pH 7.4. Use MES buffer for example; phosphate buffers can be used but reduce conjugation efficiency, although this effect can be overcome by adding more EDC. Avoid using bufffers like Tris, Glycine, acetate, citrate,...! The activated FluoProbes reacts with hydrazide, yielding the right conjugate, but also with amines; Thus in most cases with proteins (that have both carboxylic acids and primary amines available) a polymerization of the molecule is possible. This can be minimized by decreasing the amount of EDC used and/or increasing the amount of used FluoProbes Hydrazide . Alternatively, the amines on the molecule to be conjugated can be blocked using Sulfo-NHS-Acetate (UP69380).

Protocol 1: CHO-bearing molecules

– Prepare a solution of meta-periodate at 20mM in 0.1M sodium acetate buffer pH5.5 This solution should be kept in the dark at 0-4°C, and used immediately. Throw away after use.
– Prepare the protein solution at 5mg/ml in cold 0.1M sodium acetate buffer pH5.5 The protein concentration can be determined by the Bicinchoninic Acid method (#UP40840A, BC Assay).
– Add 1 ml of periodate solution to 1 ml of protein solution. Mix and incubate for 5min at 0-4°C
Remark: the ratio and incubation time should be optimized depending on the protein nature and concentration.
– Dessalt the protein by dialysis or gelfiltration in 0.1M sodium acetate buffer pH5.5
Fractions containing the labeled protein can be identified by measuring the absorbance at 280nm, or any other mean, and pooled.
– Prepare a Hydrazide-FluoProbes solution at 40mM in DMSO.
– Add 250µl of Hydrazide- FluoProbes solution to 2 ml of protein solution. Mix and incubate for 2H at room temperature.
– Dessalt the labelled protein by dialysis or gelfiltration with PBS (NaCl 150mM, phosphate 10mM pH7.4).
Fractions containing the labelled protein can be identified by BC Assay #UP40840A, or any other means and pooled.
– Labelled antibodies can be stored in PBS + 0.1% NaN3 and 50% glycerol at –20°C.

Protocol 2: COOH-bearing molecules

– Prepare the protein solution at 5mg/ml in 0.1M MES (2-N-morpholino-ethanesulfonic acid) pH5.5
– Prepare a 50mM solution of Hydrazide FluoProbes in DMSO
– Add 25µl of FluoProbes-hydrazide to 1ml of protein solution. Mix.
– Prepare a 10mg/ml solution of EDAC (#UP52005) in 0.1M MES pH5.5. Use immediately
– Add 12.5µl of the EDC solution. Mix and incubate overnight at room temperature under constant agitation.
– Dessalt the labelled protein by dialysis or gelfiltration with PBS (NaCl 150mM, phosphate 10mM pH7.4).
Fractions containing the labelled protein can be identified by BC Assay #UP40840A, or any other means and pooled.

Related products and documents

- Sulfo-NHS-Acetate #UP69380A
- SFB #M11771
- EDAC #52005A
- Reducers: DTT #UP284250, TCEP #UP242214
- Desalting: UptiSpin filters; Gelfiltration G-25 columns # 84874
- PBS buffer #UP68723A
- FluoProbes labeling agents: See selected most popular and remarkable labels, BioSciences catalogue p.B56.

Other derivatives are available, incl. amino-, carboxy-, Succinimidyl-, Azide, (strept)avidin, secondary antibodies, some specific probes such as Annexin, Phalloidin, ... or any other on custom labeling. FluoProbes® Protein labeling Kits
Fluorescent labeling of proteins to analyze in electrophoresis (2D-gel PAGE): NT-2D.

Ordering information

For any information, please ask : Fluoprobes / Interchim; Hotline : +33(0)4 70 03 73 06

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