
# CY$_{anine}$ Hydrazide

*CY$_{anine}$ fluorophores for labeling biomolecules*

## Introduction

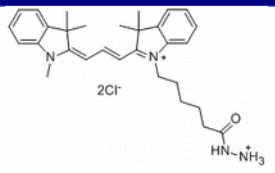
A variety of **CY$_{anine}$ dyes** has been used to label proteins, nucleic acids and other biomolecules for fluorescence techniques (imaging, biochemical analysis). They replace advantageously the conventional fluorochromes such as Fluorescein (FITC) and rhodamines (TRITC, RRX).

**CY$_{anine}$3** can replace orange-fluorescent dyes, like Tetramethylrhodamine (TRITC). Cy$_{anine}$3 is one of the most broadly used fluorophores which can be detected by various fluorometers, imagers, and microscopes. Due to inherently high extinction coefficient, this dye is also easily detected by naked eye on gels, and in solution. See also alternative superior dye: FluoProbes547H.
**CY$_{anine}$3.5** can replace SulfoRhodamine 101.
See also alternative superior dye:FluoProbes594.
**CY$_{anine}$5** can replace far red red fluorescent dyes.
During last years, CY$_{anine}$5 flurophore has become an incredibly popular label in life science research and diagnostics. Fluorophore emission has maximum in red region, where many CCD detectors have maximum sensitivity, and biological objects have low background. Dye color is very intense, therefore quantity as small as 1 nanomol can be detected in gel electrophoresis by naked eye. See also alternative superior dye: FluoProbes647H
**CY$_{anine}$5.5** can replace near infrared fluorescent dyes.
See also alternative superior dye: FluoProbes682.
**CY$_{anine}$7** is a near infrared red fluorophores used in in vivo imaging applications.
See also alternative superior dye: FluoProbes752.
**CY$_{anine}$7.5** is a near infrared red fluorophores used for *in vivo* imaging applications.
See also alternative superior dye: FluoProbes800.

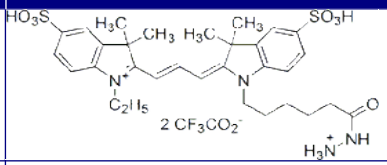
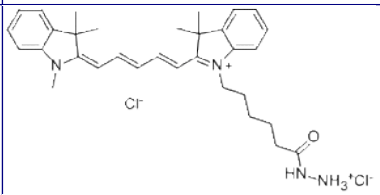
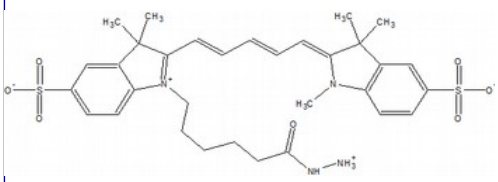
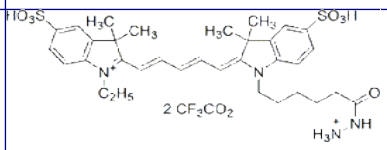
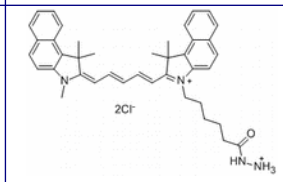
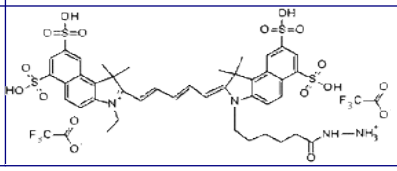
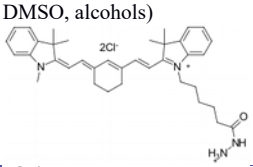
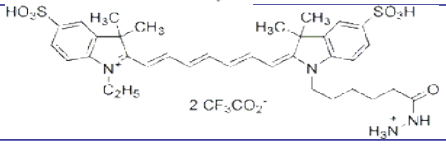
**Sulfo – CY$_{anine}$ dyes** are water-soluble form of the CY$_{anine}$ dyes. DiSulfonated forms are the most classic, but some tri- and tetra-sulfonated forms are available as well, for even higher hydrosolubility.

## Products Description

The table below gives main physical and fluorescence characteristics of the activated dyes

| **Product name** cat.number/qty* | **MW** (g·mol$^{-1}$) **+added MW** | **λ abs./em.** (nm) | **mol. abs.** (M$^{-1}$cm$^{-1}$) | Comment, structure |
|---|---|---|---|---|
| **CY$_{anine}$3 – HydrAzide** FP-SJH870, 1mg | 543.59 | 555 / 565 | 150 000 QY : 0.31 |  |

FT-LQV050

| **Product name** cat.number/qty* | **MW** ($g \cdot mol^{-1}$) +added MW | **λ abs./em.** (nm) | **mol. abs.** ($M^{-1}cm^{-1}$) | Comment, structure |
|---|---|---|---|---|
| **DiSulfo-CY$_{anine}$3 – HydrAzide** FP-LQV050, 1mg | 872.85 | 555 / 565 | - |  |
| **CY$_{anine}$3.5 – HydrAzide** Inquire, 1mg | | 591 /604 | 116 000 QY: 0.35 | |
| **CY$_{anine}$5 – HydrAzide** FP-IO2490, 1mg dark blue powder / solution Significant solubility in water, good in polar organic solvents (DMF, DMSO, alcohols) | 569.61 | 646 / 662 | 250 000 QY: 0.2 |  Also available: CY$_{anine}$5 Boc-hydrazide |
| **Sulfo-CY$_{anine}$5 – HydrAzide** FP-1C4681, 5mg Soluble in DMSO, water | 656.8 | 646 / 664 | 250 000 QY: 0.28 |  |
| **DiSulfo-CY$_{anine}$5 – Hydrazide, 2 $CF_3CO_2$- salt** FP-LQV110, 1mg | 898.89 (672.85 without salt) | 649 / 665 | |  |
| **CY$_{anine}$5.5 – Hydrazide** FP-WZE110, 1mg | 669.74 | 673 / 707 | 209 000 QY : 0.2 |  |
| **Tetra-Sulfo-CY$_{anine}$5.5 – Hydrazide** FP-LQV300, 1mg | 1159.13 | 677 / 701 | |  |
| **CY$_{anine}$7 –Hydrazide** (M) FP-WZE130, 1mg | 635.70 [+544.8] | | | moderate solubility in water, good in polar organic solvents (DMF, DMSO, alcohols)  |
| **Sulfo-CY$_{anine}$7 – HydrAzide** FP-LQV270, 1mg | 924.92 | 749 / 776 | |  |
| **CY$_{anine}$7.5 – HydraAzide** FP-WZE150, 1mg | | 788 / 808 | 223 000 | |

**Storage:** –20°C, protected from light (M)

# 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.

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

– 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):

$$R\text{-}COOH + EDAC + Hydrazide\text{-}FluoProbes \rightarrow R\text{-}CO\text{-}NH\text{-}NH\text{-}CO\text{-}(CH2)4\text{-}\ FluoProbes$$

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 ($NaIO_4$). 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 pH 5.5 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 pH 5.5 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 pH 5.5

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 fo 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).

## Related products

* Other $CY_{anine}$ dyes functionalized by **NHS** (BB7493), **Maleimide** (JO6660), **Azide** (HO7250), **Alkyne** (1A6320), **Hydrazide** (LQV050), **DBCO** (DQP790: CycloAlkynes, for strain-promoted Click reactions), **Amino** group (CY3AM0), **Carboxyl** group (CY3CA0). **3Dye 2D DIGE ($CY_{anine}$2/$CY_{anine}$3/$CY_{anine}$5) labeling kit** (EV0870)

* Related labels: Superior **FluoProbes** **fluorescent dyes**
- activated by –NHS (list), i.e. FP488-NHS #BA68000 - activated by –Azide, i.e. FP488-Azide #YE4970

* Accessory reaction reagents: • EDAC (UP52005A, FP-WU5580) • MES Buffer, (14035)

# Ordering information

Catalog size quantities and prices may be found at www.interchim.com/. Please inquire for bigger quantities and for any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06