FT-EV0870

3Dye CY$_{anine}$2/3/5 fluor Labeling Pack

CY$_{anine}$ fluorescent labeling of amine-containing biomolecules for Multiplex differential analysis.

Product Description

Product name cat.number	content
3Dye CY$_{anine}$2/3/5 Fluor Labeling packs	
(K) EV0870, 5nmol kit	Contains: 5nmol of each CyFluor-NHS, and 1ml of DMF
EV0871, 10nmol kit	Contains: 10nmol of each CyFluor-NHS, and DMF
EV0871, 25nmol kit	Contains: 25nmol of each CyFluor-NHS, and DMF

Introduction:
The 3Dyes Labeling kit contains a set of optimized fluorescent reagents that allow basic and clinical research scientists to easily, conveniently and reproducibly label total proteins, or any other amine-containing biomolecules for differential analysis.
The 3 CY$_{anine}$ fluor dyes are selected to match both molecular weight and ionic charges after labeling of biomolecules. This ensures that proteins are modified in similar ways by the 3 dyes, making their pattern not dependent on the dye. That is taken in good account in techniques where similar samples should be compared, for example comparing a sample from a normal and a pathologic patient, comparing normal cells to drug-treated cells, comparing the effect of different culture conditions or cytokine effects,.. Such differential analysis is performed by electrophorosis and fluorescent detection techniques, of cells lysates (cultured in suspension, adhering, or from solid tissue and tumor biopsies) as well as other samples (protein fractions, rec.proteins).

Directions for use

Storage/handling:

Transportation: at room temperature for up to 3 weeks.

Storage: at -20°C in the dark, up to 24 months after reception. Avoid prolonged exposure to light. Desiccate.

Unfreeze completely before opening vials, and purge with argon before closing.

The pack contains lyophilized dyes, which should be re-constituted prior to use with labeling grade DMF (included in the pack).

● Fluorescent labeling

The labeling of proteins involves the reaction of Succinimidyl group (NHS) with amino groups from proteins or other molecules. Resulting labeling does not affect the ionic charge of proteins.

● Proteins can be labeled for 30-60min at room temperature, with 1:1 to 30:1 ratio of dye to protein, at physiological pH to mildly alkaline conditions, depending on application.

LIFE SCIENCES
interchim 211 bis, avenue JF Kennedy BP 1140 - 03100 Montluçon Fax +33 4 70 03 82 60 Hotline +33 4 70 03 73 06 • interbiotech@interchim.com

Note: if the sample is obtained by lysis of cells or fractionation, the used buffers should be compatible with NHS-labeling, hence devoid of amine compounds.

Optimal labeling for differential analysis performed with minimal labeling ratio is typically performed **for 30min on ice at pH 8.0 or pH 8.5**, using buffer such as 7-8 M urea, 0-2 M thiourea, 4% CHAPS, pH 8.0 à 8.5 (plus 5mM Magnesium acetate for certain samples types), and using **400 pmol of each dye per 50 µg of protein**.

Note: check the pH of you protein samples, i.e. cell lysate. As pH affects the kinetic and extend of labeling, significant differences of labeling and sample analysis pattern may be observed when labeling at different pH, i.e. pH 7.3 versus pH 8.0. Furthermore, above pH 8.5, unspecific labeling may occur. Hence the pH of the protein samples should be properly adjusted (check 2µl on a pH indicator paper; add suitable alkali to reach desired pH (8.5)). Protein concentration should also be determined (BCA assay)
Note: Lower labeling ratio (200pmol/50µg protein) may be acceptable for some applications, while up 10-fold ratios are needed for saturation labeling of scarce samples . However, the MW of labeled proteins may be significantly affected at saturation protocols.

Protocols can be found in the literature.

●Cell surface proteins can be labeled [1].

Wash 5–10 .10^6 cells by centrifigation and resuspension to remove culture medium
and resuspend the cell pellet in 200 µl ice cold labeling buffer (HBSS pH 8.5, 1 M urea).

Label the cells with 600 pmol CY_{anine} dyes for 20 minutes on ice in the dark.

Quench the reaction by adding 20 µl 10 mM lysine. Incubate for 10 minutes.

Wash the surface-labeled cells twice by resuspension in 500µl HBSS pH 7.4, followed by centrifugation at 800g at 4°C for 2 minutes.

CY_{anine}-labeled cells can then by lysed and fractionnated.

● Different usages of the 3Dye CY_{anine} Fluor dyes

The 3 provided dyes are used to label separately one sample each. The labeled samples can be analyzed separately or combined in several ways for multiplex detections.

For differential analysis, to increase accuracy of samples comparison, $CY_{anine}2$ is typically used to label a reference protein that is further used as an internal control in analysis: the $CY_{anine}2$ labeled control protein is added to samples before analysis, and in particular to a 1:1 combination of $CY_{anine}3$ and $CY_{anine}5$ labeled samples for subsequent simultaneous detection at 3 wavelengths (multiplexing).

label sample1 by $CY_{anine}3$ Fluor (orange)
label sample2 by $CY_{anine}5$ Fluor (red)
label reference or control sample by $CY_{anine}2$ Fluor (green)
mix $CY_{anine}2$-reference sample (internal control) + sample1-$CY_{anine}3$ Fluor + sample2-$CY_{anine}5$ sample and analyze

The internal control ($CY_{anine}2$) is an effective way to minimize gel-to-gel variation and it significantly increases accuracy and reproducibility:

When the $CY_{anine}2$-labeled control is the reference (untreated protein sample) of treated sample1 and sample2 (i.e. drug treated cells, stimulated cells, different condition of culture,…), one can measure 'normal' proteins kept, lowered or increased by treatments (spots detect for both $CY_{anine}2$ and $CY_{anine}3$ or $CY_{anine}2$ and $CY_{anine}5$), and new proteins induced by treatment 1 or 2. The fluorescence difference accounts for the real protein concentration difference.

When used in different gels, the $CY_{anine}2$-labeled internal control allows aligning protein migration profiles from different gels, and eventual correcting differences fo fluorescence intensity between gels, increasing the accuracy of protein spots identity and of their abundance.

Operating so, differential analysis of samples is improved by simultaneous co-separation (i.e. 2D electrophoresis) and detection (multiplex imaging) of samples, and furthermore by imaging analysis with suitable software.

LIFE SCIENCES
interchim 211 bis, avenue JF Kennedy BP 1140 - 03100 Montluçon Fax +33 4 70 03 82 60 Hotline +33 4 70 03 73 06 • interbiotech@interchim.com

FT-EV0870

● Instrument compatibility:

- The CY$_{anine}$ Fluor dyes are compatible with all imagers capable of detection of CY$_{anine}$2, CY$_{anine}$3, and CY$_{anine}$5 fluorophores.

Laser wavelength and emission filters:

Dye	Laser	Emission filter (nm)
CY$_{anine}$2	Blue 2 (488nm)	520BP40 (green)
CY$_{anine}$3	Green (532nm)	580BP30 (orange)
CY$_{anine}$5	Red (633nm)	670BP30 (far red)

- Open Source imaging systems have been developed by academic groups : Flicker, JvirGel,

References

- **Dautel F**. *et al*., Large-scale 2-D DIGE studies - guidelines to overcome pitfalls and challenges along the experimental procedure, *JIOMICS*, Vol 1:Issue1:170-179 (2011) Article
- **Sorzano CO** *et al*., Elastic image registration of 2-D gels for differential and repeatability studies, *Proteomics* 8(1):62-5 (2008) Abstract

Related products and documents

CYanine2-NHS #LV2330 (for higher ratio labeling)
All other CYanine – NHS (FT-BB7493)
Other succinimidyl activated fluorescent dyes: CYanines, FluoProbes
+
Protein dosage: BC Assay protein determination kit #40840A
Protein extraction reagents: see catalog BB002c or inquire a solution designed for 2D D.I.G.E analysis #FY3340

See BioSciences Innovations catalogue and e-search tool.

Ordering information

Catalog size quantities and prices may be found at 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

 Rev.M0601E

LIFE SCIENCES
interchim 211 bis, avenue JF Kennedy BP 1140 - 03100 Montluçon Fax +33 4 70 03 82 60 ■ Hotline +33 4 70 03 73 06 • interbiotech@interchim.com ■