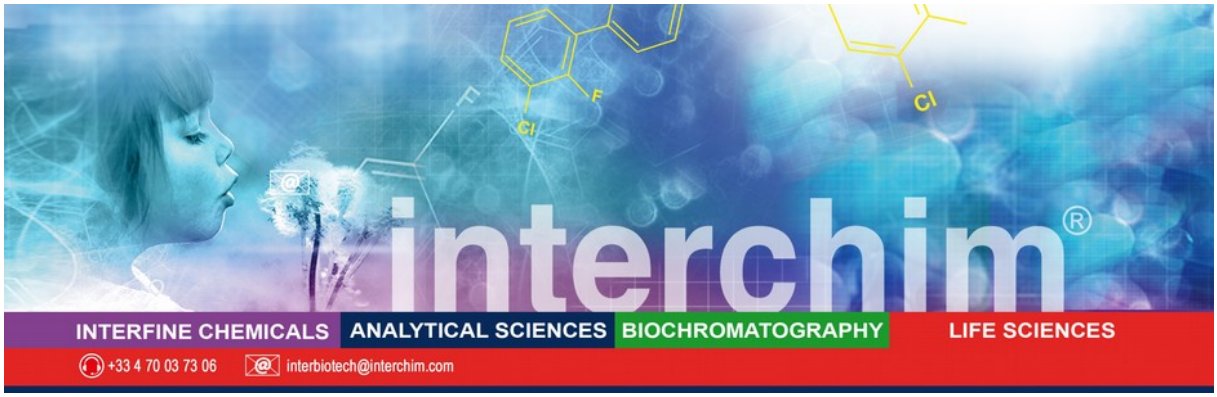
FT-BE3750



FluoProbes® Protein Labeling Kit

Label easily and quickly your antibodies or proteins with great FluoProbes® dyes (5x1mg protein)

Product Description

Dye cat.number	MW ($g \cdot mol^{-1}$)	λ_{exc}\λ_{em} max. (nm)	Comments	EC (ε) $M^{-1}cm^{-1}$	Dye qty per vial	Solvant volume
FluoProbes® 350 FP-1J8690, 5rxns	874,10	353/432	Useful blue label for multi-color detections.	19 000	50 nmol	25 µl
FluoProbes® 405 FP-1G6210, 5rxns	792.66	400/423	Useful blue label for multi-color detections.	32 000	50 nmol	25 µl
FluoProbes® 415 FP-BS5600, 5rxns	573.56	418/465	Useful blue label for multi-color detections. Suits AMCA filters.	34 000	50 nmol	25 µl
FluoProbes® 481XXL FP-CV3240, 5rxns	727.75	515/650	Large stock shift, allowing multi-color detections with a single wavelength excitation at 488 nm.	50 000	50 nmol	25 µl
FluoProbes® 490 FP-JO0910, 5rxns	1011.20	491/515	Photostable, high brightness, pH-stable fluorescence. Suits FITC and Cy™2 filters	73 000	50 nmol	25 µl
FluoProbes® 495-X5 FP-AX1350, 5rxns	623.02	494/519	The conventional standard FITC with improved chemistry (longer spacer) for superior results.	70 000	50 nmol	25 µl
FluoProbes® 547H FP-1Q3930, 5rxns	1040.06	550/575	More hydrophilic than FP547. For highest dye:protein ratio. Suits Rhodamine, TRITC and Cy™3 filters	150 000	50 nmol	25 µl
FluoProbes® 554 FP-BU7240, 5rxns	677.16	551/572	Suits Rhodamine filters	100 000	50 nmol	25 µl
FluoProbes® 590 FP-BC2940, 5rxns	816.96	580/599	A great bright red fluorescent label Equivalent to TR/SR101 but with improved feature (extended and hydrophilic spacer).	120 000	50 nmol	25 µl
FluoProbes® 594 FP-IV5670 5rxns	1078.10	594/615	Brightest red fluorescent label with excitation at 594 nm	92 000	50 nmol	25 µl
FluoProbes® 634 FP-CG6750, 5rxns	1066.1	637/658	Excited by 633 nm He-Ne lasers High water solubility	200 000	50 nmol	25 µl
FluoProbes® 647H FP-1I7620, 5rxns	1066.10	655/676	More hydrophilic than FP647. For highest dye:protein ratio. Suits Cy™7 and Cy™5 filters	250 000	50 nmol	25 µl
FluoProbes® 682 FP-BE8280, 5rxns	950.03	690/709	Highly hydrophilic	140 000	50 nmol	25 µl

Composition of a kit

FluoProbes® dye:	5 vials of succinimidyl (SE) dye (50nmol each)
Component a:	2 vials of Solvent for SE-FluoProbes® dye (DMSO)
Component b:	5 vials of Labeling Buffer reagent (to reconstitute with 1ml water / each) ($NaHCO_3$)
Component d:	5 Reaction microtubes, 0.5ml
Component e:	10 Centrifuge Filter columns (e1) with 10 wash tubes (e2) and 10 collection tubes (e3)

Storage: Short term: +4°C (<6months) . (L) Components a to e may be stored at room temperature. SE-dye may be stored for long term at –20°C. Protect from light and moisture.

Introduction

FluoProbes® Protein Labeling kits offer an advantageous alternative to home-made labeling and other commercial labeling kits. These kits perform easy and efficient labeling of antibodies, enzymes, or other proteins with molecular weight higher than 25 kD, and employs our superior FluoProbes labels. The FluoProbes® kit uses a Succinimidyl ester dye to create a covalent bound with proteins. The conjugate is desalted with convenient Centrifuge Filter Columns.

FluoProbes® benefits	*Quick facts*
• Faster and easier procedure	60-90min
• Scalable	for quantities 100µg-1.5mg
• More economic per labeling	Compare price per run
• Available with >50 FluoProbes® dyes	Including great FP labels and conventional labels
• Excellent recoveries	>90% (Hu IgG / standard protocol)

Directions for use

This standard protocol r is designed for 1mg antibody at ca 5mg/ml [a], but works well down 100µg Ab at 0.5-20mg/ml concentrations, as well as for any other protein of similar mass, provided it is adapted (refer to notes [1] [5a]).

1/ Prepare the protein solution at 5mg/ml.

-Proteins in solution with suitable buffers as PBS, Carbonate, Borate,... can be used directly.
CAUTION: Protein solution should be free of amines and ammonium sulfate.[caution note, 1b]

-Lyophilized proteins may be simply dissolved in suitable[1b] buffers.

-If required, dilute or concentrate to 5mg/ml for standard conditions. 2-10mg/ml work fine also (see note[1c]).

-Up to 15 nmol of protein can be labeled using one vial (50nMol) of reactive dye (equivalent to 2,18 mg IgG, 1,0mg BSA or avidin and 0,68mg ovalbumin).

Refer below to notes [1a-c] for special cases, optimizations and required adaptations such as proteins with MW >25 000Da, lower or higher concentrations or quantity, unsuitable buffer or interfering substances.

2/ Prepare the conjugation/labeling buffer:
Dissolve one vial of Component b with 1 mL of deionized water (vortex). Unused buffer may be kept at 4°C for several days.

3/ Add 20µl of labeling buffer (step 2) to 100µl of protein solution (5mg/ml - step 1) in a provided microtube.
Lower or higher protein volumes can be used, up 500µl in provided tubes. Use your own tubes for higher volumes (adaptations desalting step (8/) if required).

4/ **Prepare the FluoProbes® reactive dye working solution** at 2mM (at a time you are ready for step 5):
Tap one vial of reactive dye to ensure no due is in the cap, and add 25µl (or 150µl for FP488 or 120 µl for FP647N) of provided solvent (component a). Mix well (vortex), eventually pipette up and down, and warm slightly the vial to achieve complete dissolution.

NB: Use this working solution [=2mM] **EXTEMPORANEOUSLY** (degrade with time: do not use 5min after preparation). Do not keep unused reagent.

5/ Add 20 µl of FP® dye solution 2mM to 0.5-1mg protein in solution in the reaction tube, and mix (vortex gently).
The quantity of dye to add should be scaled with protein quantity, and may be adjusted (calibrated) regarding protein concentration, or optimization of Dye/Protein ratio (D/P) for demanding applications. Refer to note [5a] .

6/ Incubate for 5min at room temperature, protected from light.
In that time, prepare desalting step 8[8].

7/ Desalt the labeled protein by gelfiltration with provided spin filter columns, or other suitable mean.

The provided Centrifuge Filters columns desalt quickly and conveniently proteins in most applications (protocol in note [7]). However, they should not be used for proteins with lower MW than 25 000Da, or may be less suited for diluted samples. Ask for other suitable desalting methods such as dialysis (i.e. CelluSep tubings), gelfiltration (provided Centrifuge Filter columns , or for larger volumes Desalt columns #UP84874) or ultrafiltration (UptiSpin concentrators).

8/ The desalted labeled protein is ready for downstream experiments.

Check eventually for **protein content** (step 9) and for the **degree of labeling** (step 10).
Be aware the labeled protein may be susceptible to alterations of its activity (especially in case of over labeling), and degradation by bacteria freeze/thawing cycles (aggregation/precipitation/cleavage). **Preservatives** can be added for storage purpose (i.e. 0.09% Azide), but should be compatible with for further uses. Labeled Abs can be stored at +4°C, or frozen for longer term (avoid repeated freezing/thawing, and protect from light!).

9/ *(facultative)* Determine the Protein content

Accurate **protein determination of proteins** can be performed by a BC Assay (#UP4080A),or Bradford assay (#UPF86400). Alternatively, protein can be estimated by absorbance measurement ($Conc._{protein} = A_{280nm}$ x 1.65 for IgGs), or accounting a 90% yield ($Conc._{protein}$ = initial protein quantity x 0.9 / total final volume).

10/ *(facultative)* Determine the **Degree of Labeling**

-Measure the absorbance of the desalted labeled protein diluted in PBS at both 280 nm [A_{280}] and FluoProbes® label maximum excitation wavelength ($\lambda_{em.max}$: see table on front page) [$A_{\lambda em.max}$]

The labeled protein should be well desalted: non-conjugated dye would biase the calculation of D/P ratio.
Use a 1cm path length spectrophotometer, or correct absorbance proportionally. Small volume cuvettes (down 50µL!) to spare sample.
To get OD in the linear range or the spectrophotometer, the dilution should reach usually 0.1-1mg/ml of protein. This can be estimated from the starting concentrations and initial/final volume considering 90% recovery.

-Calculate the degree of labeling (D/P=number of dye molecule / protein molecule, or 'molar Dye:Protein ratio'):

$$D/P = \frac{[\ A_{\lambda em.max}\ ] \times EC_{protein}}{[\ A_{280} - (A_{\lambda em.max} \times 0.2)\ ] \times EC_{dye}}$$

$A_{\lambda em.max.}$: Measured Absorbance at $\lambda_{em.(dye)}$ (wavelength of maximum excitation for the used fluorochrome)
Note: when the protein showed significant absorption at the $\lambda_{abs.max}$ of the dye, a correction factor ($CF_{prot.}$ should be determined previously and applied in Abs calculation:
$A_{\lambda em.max}(corr.) = A_{\lambda em.max} - (A_{280} \times CF_{prot})$. with $CF_{prot.} = A'_{\lambda em.max[unlabeled\ protein]} / A'_{280nm[unlabeled\ protein]}$
A_{280}: Measured Absorbance at 280nm (significant of protein)
EC: molar extinction coefficient at $\lambda_{abs.max}$ of the protein ($EC_{prot.}$) and of the dye (EC_{dye}: see the table of the front page of this technical sheet).
0.2: average correction factor accounting for the absorption of the dye at 280nm (CF_{dye}).

The calculated D/P ratio is to be used as a relative indication of proper labeling, or to compare different labeled conjugates with a same dye. i

Notes

The above standard protocol is designed for IgG antibodies, but works well for any protein or amine-containing biomolecule. Following are some technical tips to adapt it if required.

Note [1a]: **Proteins of lower MW than 25 000Da** can be labeled, however desalting should not be performed with provided columns, but by dialysis with a MWCO 10KDa or below. Please inquire.
Some **difficult proteins** have a particular Lys content, ionic charge or hydrophobic pattern, that may require to calibrate the Dye/Protein ratio (also needed when a defined coupled ratio is required in your application). Refer to note [5a].
Note [1b]: The labeling accommodates most **physiological buffers**, including PBS, Borate, Hepes and Carbonate, provided they are **devoid of amine** compounds (i.e. no Tris, Glycine). Proper labeling can be performed in presence of low concentrations of sodium azide 0.01% or thimerosal (<1mM), but glycerol above 20% and ammonium ions reduce labeling efficiency. Labeling can be performed at various pH ranging for 7.5 (slower reactivity) to 9.5 (faster reactivity, but also higher competition of hydrolysis, and eventual alteration of protein). pH 8.5 is recommended.
As varying buffers may result in variable labeling efficiency, FluoProbes recommends using our labeling buffer/step3.
Exchange of buffer or rapid desalting may be required, and performed by dialysis (i.e. CelluSep tubings), gelfiltration (Desalt columns #UP84874) or ultrafiltration (UptiSpin concentrators, or provided Centrifuge Filter columns).
Note [1c]: **lower or higher protein concentrations** than 5mg/ml concentration work fine also. The incubation ratio of dye/protein should however be adjusted when protein concentrations are down 0.5-2mg/ml or up 10-20mg/ml. Refer to note [5a]. Down (50)µg of proteins (useful for monoclonal abs) and up 2mg can be labeled.

Note [5a]: **Added dye volume adjustment / Optimization of Dye/Protein ratio (D/P)**
The standard volume of activated dye to be added is sat to 20µL with a standard molar incubation ratio (D/P) sat to 6 Dye/Protein during incubation for 100µl IgG antibodies at 3-10mg/ml. It is expected to yield 2-4 coupled dyes per IgG. It also suits proteins sized 25KDa to 1000KDa. However, this standard dye quantity (or volume) should be eventually adjusted depending on protein quantity, protein concentration, protein species/nature/size, or when higher or lower coupled D/P is desired to optimize the activity of labeled protein.

You can **adjust the volume of dye** to prepare and to add in reaction mixture, from the standard value [20µl] with your specific values (desired incub.D/P, molar protein concentration, protein volume):

- $Volume_{dye} = C_{m.protein} \times V_{protein} \times 1000 / C_{act.dye} / MW_{protein} \times MR$.

Where $C_{m.protein}$ is the mass concentration of protein solution in mg/ml
$C_{act.dye}$ is 2 µMol/mL, the molar concentration of activiated dye
$V_{protein}$ is the volume of protein to be added in reaction. The recommended volume is 100µL.
$MW_{protein}$ is the Molecular Weight of protein. For most IgGs, this is 145 000.
MR is the Molar Ratio of activiated Dye to Protein (D/P) in the reaction mixture. This will NOT be the end Molar Ratio of conjugated dye-protein, which is substantially less. We recommend a MR of 5-6 for labeling IgGs.

-lower protein concentration effectively decrease labeling yield, thus the molar ratio of dye/protein may be increased for protein concentration down 0.5-2mg/ml of protein, and or slightly decreased for 10-20mg/ml.
-non-globulin proteins, oligopeptides, basic or acidic proteins may require different ratio of dye to yield a same coupled D/P ratio. Higher MW usually requires higher molar D/P ratio, but this often leads to have a similar mass D/P. But the same D/P ratio will not give the optimal labeling of different proteins, even within antibodies from different species, classes or isotypes (monoclonals). Finally thus, a calibration of the incubation D/P ratio is useful, and required in demanding applications, to yield an optimal coupled D/P ratio for optimal labeling in each downstream application.

Note [7]: Desalting operating with Centrifuge Filters (Gelfiltration)
7a/ Prepare Filter spin columns:

-Take 2 spin columns (components e) to desalt the standard 140-180µl of labeling mixture

-Gently tap or briefly vortex the columns to resuspend gel and remove bubbles.

-Open the upper stopper (cap) and Remove the bottom caps and the the top caps. Let the dry gel settle.

-Drain off interstitial fluid by centrifugation of the columns in a wash tube (component **e2)** at 1000g for 2 minutes. The flow stops on its one when the upper reservoir is empty. If there is a drop at the end of the columns, blot it dry.

Process the samples with the next minutes.

An horizontal of winging-bucket rotor provide maximum yield and efficiency.
If you use a fixed-angle microcentrifuge (provide acceptable performance, and save time), keep track of the position of the columns using the operation mark molded into the columns.
If you ise a variable speed microcentrifuge, do not use the pulse buttom (overrides the speed settings).
If you are not sure of the g-force generated by your centrifuge, calculate speed with the formula:
rpm = square (RCF / 1.119 x 10-5 x r) , where rpm is revolutions per minute, RCF is relative centrifugal force, r is the radius (cm) measured from centerof spindle to bottom of rotor bucket.

7b/ Desalt sample:

-Pipette the labeling mixture (<90µL*) gently** onto the top of an equilibrated column.

* Don't process more than 90µl per column. One typically deposit each half of 140-180µL of labeling mixture into 2 spin columns that equilibrate each other in the centrifuge. For bigger volumes, please ask for desalting columns #UP84874 rather to desalt sequentially multiple 90µl aliquots that is fastidious (suppose to regenerate/equilibrate the column with >4ml PBS between each desalting process).

** Operate up to light, dispense slowly at the center on the gel to avoid gel suspension OR TO contact with the column sides (this can reduce the efficiency of purification). But do not waste time until elution step.

-Absorb the drop at the outlet of the column, and place the column into the collection tubes (component **e3**) then place both into the rotor.

Maintain proper orientation if using a fixed-angle rotor: the highest point of the gel should point towards the outside of the rotor.

- Spin the columns and collection tubes at 1000g for 2 minutes. Discard the spin columns.
  Recover the purified protein conjugate (~130µL from both collection tubes/columns.

The last process can be repeated with a small volume of buffer (50-100µL) for increased recovery.

Related products & Ordering information

- PBS buffer, 1 tablet/100 ml, 307157
- Desalting columns (4 or 10ml gel), UP84874
- FluoProbes® 547H Goat anti-Mouse IgG, FP-SB4000
- FluoProbes® 547H Goat anti-Rabbit IgG, FP-SB5000
- FluoProbes® 647H Goat anti-Mouse IgG, FP-SC4000
- FluoProbes® 647H Goat anti-Rabbit IgG, FP-SC5000

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability. shipment conditions).
For any other information, please ask Fluoprobes® / Interchim; Hotline : +33(0)4 70 03 73 06, info@fluoprobes.com .

Rev. G09E