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Technical Information

Flamma® Fluors Protein Labeling Kit

Overview

Flamma® Fluor Protein Labeling Kits from BioActs are designed for efficient labeling of antibody or protein with fluorescent materials. Due to strong absorption, high fluorescence quantum yield, and high photostability, Flamma® Fluor Vinylsulfones are selected as reactive dyes, and they maintain good fluorescence activity and stability after conjugated to biomolecules, allowing detection of low-abundance biological structures with great sensitivity. Vinylsulfone reactive group is selectively bind to the primary amines of proteins to create efficient dye-protein conjugates. The Labeling Kits are optimized for labeling 1 mg of antibody per reaction, and contain everything needed to perform the conjugation. Flamma® Fluors Protein Labeling Kits enable to perform the entire step from labeling to purification.

Table 1. List of Flamma® Fluors Protein Labeling Kits

Cat. No.	Product	Ex (nm)	Em (nm)	Emission Color	Scale
XPL1104	Flamma® 552 Protein Labeling Kit	550	565	Orange	1 mg × 3 labeling reaction
XPL2104	Flamma® 648 Protein Labeling Kit	648	663	Far-red	
XPL3104	Flamma® 675 Protein Labeling Kit	675	691	Near-IR	
XPL4104	Flamma® 749 Protein Labeling Kit	749	774	Near-IR	
XPL5104	Flamma® 774 Protein Labeling Kit	774	806	Near-IR	

Table 2. Components of Flamma® Fluors Protein Labeling Kit

Part No.	Contents	Amount	Storage	Remark
ZPL-0001	Component A, Flamma® Fluors Vinylsulfone dye	3 vials	2~6 ℃	Spin-down before use
ZPL-0002	Component B, 10 × PBS buffer	15 mL	2~6 ℃	
ZPL-0003	Component C, 10 × Reaction buffer	500 µL	2~6 ℃	
ZPL-0004	Component D, Purification column	3 pcs	4~30 ℃	Shall not be frozen
ZPL-0005	Component E, Column adapter	1 pcs	-	
ZPL-0006	Component F, Disposable pipette	3 pcs	-	

General information

Flamma® Fluors protein labeling kits are optimized for the fluorescence labeling of IgG antibody. The optimal amount of antibody per reaction is 1 mg (~140 kDa) in 2 mg/mL solution. If the protein preparation condition does not match with above, please refer the "Tips and Troubleshooting" section of this document. However, protein labeling kits can be used for fluorescence labeling of a variety of other proteins. Please check "Tips and Troubleshooting" for efficient labeling. Prior to labeling, the antibody/protein should be purified and dissolved in ammonium ions or amine-free buffer. If the being labeled antibody/protein is dissolved in an unsuitable buffer such as Tris or glycine, replace the buffer with

phosphate buffered saline (PBS) by dialysis.

Protocol

Labeling

1. Prepare 0.5 mL of antibody/protein solution in 2 mg/mL concentration at room temperature prior to the reaction.
   * If the amount of antibody/protein is less than above, refer "Tips and Troubleshooting" in this document.
2. Add 50 µL of "Component C, 10 × Reaction buffer" to above antibody/protein solution and vortex the mixture.
3. Transfer the [antibody/protein solution in step 2] to "Component A, Flamma® Fluors vinylsulfone dye" vial, cover it with cap, and vortex reaction mixture.
4. The step 3 reaction mixture is stand for 2 hours at room temperature under dark.
   * Note: in general antibody labeling condition, Vinylsulfone dyes proceed about 65% of labeling at 1 h, 85% at 2 h, 90% at 4 h, and the maximum labeling can be achieved at 6 h.

Purification of conjugates

1. As shown in Figure 1, Connect “Component E, Column Adapter” to a 50 mL conical tube, and stand the assembly in a tube rack.
2. Open the upper lid of “Component D, Purification column”, cut along the perimeter of the lower faucet cap, mount it onto the conical tube assembly, and drain internal stock liquid through the bottom faucet.
   *be careful not to spill resin inside Component D.
3. While the stock liquid is drained, dilute 5 mL of “Component B, 10 × PBS buffer” by 10 fold to make 1 × PBS buffer in a separate 50 mL conical tube.
   *For dilution, use distilled water of room temperature.
4. After all of the stock liquid is drained, dismount 50 mL conical tube, discard the liquid inside the tube, and reinstall it to complete the construction of the purification system
5. Treat 5 mL of 1 × PBS buffer to “Component D, Purification column” by using “Component F, Disposable pipette” and drain the buffer, repeat the process several times to make the column to equilibrate with 1 × PBS buffer.
6. When labeling reaction (**Labeling**, step 4) is complete, the reaction mixture is carefully loaded to “Component D, Purification column”, and fluorescent dye conjugate is separated with resin column by eluting with 1 × PBS buffer.
7. Usually, fluorescence conjugated antibody/protein will come out first, and unreacted antibody/protein will come out later.
   *Be sure not to be contaminated by unreacted or free dyes.

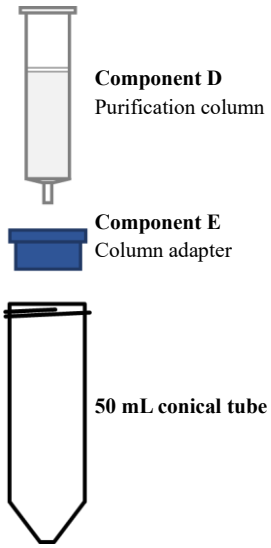

Figure 1. purification system

Calculation of Dyes/Protein ratio

Absorbance measurement

Measure the absorbance of collected fluorescence conjugated antibody/protein solution from purification step 7 and obtain the absorbance value at the maximum Abs. wavelength, which stated in Table 3.

Table 3. Optical Properties of Flamma® Fluors

Labeling Kit	$Abs._{max}$ (nm)	Extinction ($cm^{-1}M^{-1}$)	CF_{280}
Flamma® 552 Protein Labeling Kit	551	150,000	0.08
Flamma® 648 Protein Labeling Kit	648	250,000	0.05

Flamma® 675 Protein Labeling Kit	675	220,000	0.18
Flamma® 749 Protein Labeling Kit	750	220,000	0.04
Flamma® 774 Protein Labeling Kit	774	200,000	0.12

Calculation of antibody/protein concentration

$$[\text{Antibody/protein concentration } \mathbf{(M)}] = \frac{[A_{280} - (A_{max} \times CF_{280})]}{\text{molar extinction coefficient of protein}}$$

*The typical molar extinction coefficient of IgG antibody is around 203,000 $cm^{-1}M^{-1}$, and that of other protein may be vary.

$$\text{Dyes/Protein ratio } (\mathbf{d/p\ ratio}) = \frac{A_{max}}{\text{molar extinction coefficient of dye} \times \mathbf{M}}$$

*The optimal d/p ratio for IgG antibody is 3 ~ 7.

Storage and handling of dye-conjugates

Dye-conjugated antibody/protein solution shall be stored under refrigerated condition in a dark. The storage period can extended to several months when 0.5 ~ 2.0 mM sodium azide was added. For longer period of storage, store the conjugate solution at -20 ℃, but avoid repeated freezing and melting. When the concentration of conjugated antibody/protein is 1 mg/mL, add 0.1 to 1.0% stabilizing protein such as BSA and store. Do not use aggregated or sediment part, and always centrifuge the stored solution before using and use supernatant only.

Tips and Troubleshooting

When the concentration of starting antibody/protein solution is less than 2 mg/mL

The maximum allowed volume for starting antibody/protein solution is 1 mL, and the lowest permissible concentration for labeling is 1 mg/mL. When labeling a dilute protein solution, use more amount the solution up to 1 mL, accordingly. However, when the concentration of protein is less than 2 mg/mL, an excessive labeling might occur.

When molecular weight of starting antibody/protein is deviant from 140 kDa

This labeling kit is optimized for labeling biomolecules with size around 140 kDa. Thus, when the weight of your starting antibody/protein is far deviant, you need to find the optimal labeling condition empirically.

When the d/p ration of labeled antibody/protein is low

When d/p ratio of labeled protein is less than 3, the labeling rate is considered as low. In this case, please check below items.

- Check the presence of primary amines in the buffer.
- Check pH value of buffer solution (avoid low pH).
- Change the buffer solution to PBS through a dialysis or desalting column
- If the concentration of protein is less than 2 mg/mL, use more concentrated solution
- In case of protein/antibody with low reactivity: add more dye or increase the labeling reaction time.

When the d/p ration of labeled antibody/protein is high

When d/p ratio of labeled protein is above 7, the labeling rate is considered as high. The higher d/p ration can cause the aggregation of fluorescence conjugated antibody/protein, decreasing bioactivity, or self-quenching effect. In this case, using less amount of dye or

reducing labeling reaction time can be considered.

Custom Labeling Service

Based on accumulated know-how and technologies, BioActs provide a wide range of custom services such as protein fluorescence labeling, organic synthesis, oligonucleotide synthesis upon customers' request. Our reliable technology has acknowledged by our clients from domestic and overseas universities, institutions, in vitro diagnostic and pharmaceutical companies and has enabled to steadily conduct their requirements. In addition, we can introduce fluorescent materials to many other compounds such as organic and inorganic compounds, drugs, hormones, polymer, peptides, proteins, antibodies, etc. We also can provide chemical and optical analytical data, along with cell and animal experiments.

Technical Support

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SDS (Safety Data Sheets) You can find SDS at www.bioacts.com, the official website of BioActs.
CoA (Certificate of Analysis) provides detailed quality information of each product. To see CoA, check the lot number written on each product's page at www.bioacts.com, when having trouble with check, contact to our technical support team