MagicLink™ Fluorescein Antibody Labeling Kit

Components

Kit Components		Product size						Storage
		Fluorescein NHS antibody labeling kit			Fluorescein Mal antibody labeling kit			
		BP-50014 (1x100 ug)	BP-50013 (3x100 ug)	BP-50012 (1x1 mg)	BP-50035 (1x100 ug)	BP-50034 (3x100 ug)	BP-50033 (1x1 mg)	
A	Active Dye	1	3	1	1	3	1	-20
B	Buffer	1	1	1	1	1	1	RT
C	Desalt column	1	3	1	1	3	1	RT
D	DMSO, 1rxn 1ml	1	1	1	1	1	1	RT
E	NaN3 3% 0.5ml	1	1	1	1	1	1	RT
Note: The kit above is designed for IgG antibodies, but works well for any or thiol containing biomolecule. Please follow same technical tips if required.								

The fluorescein labeling kit is available in two formats, NHS and maleimide. Other formats are available upon request.

Overview

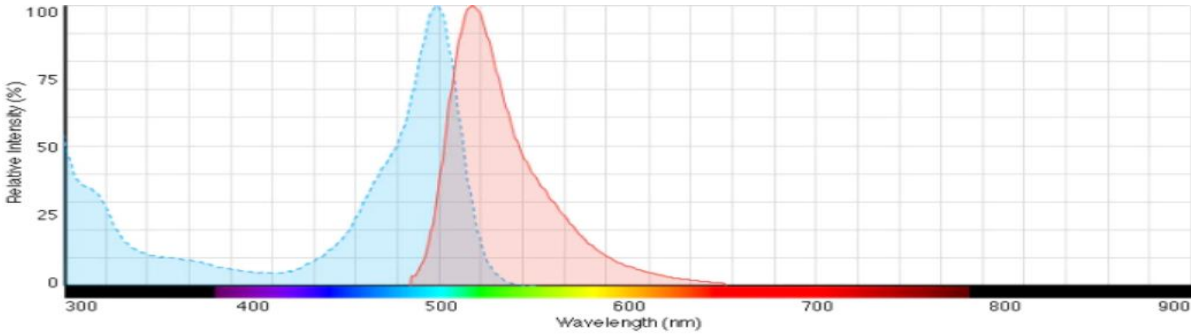
The fluorescein labeling reagents and kits are high-performance derivatives of fluorescein dye, activated for easy and reliable labeling of antibodies, proteins and other molecules for use as fluorescent probes.

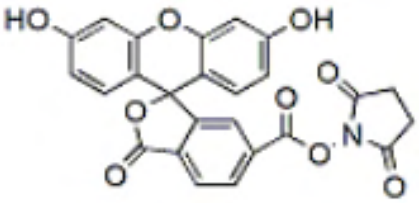
Fluorescein dyes are used in wide-ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence-based assays such as western blotting and ELISA. Fluorescein PEG4 NHS are reactive toward primary amine groups on proteins, peptides and other biomolecules, while fluorescein-5-maleimide reacts with free sulfhydryls on cysteine residues. Most activated fluorescein derivatives are mixture of isomers with reactive groups attached at the 5- and 6-positions of the bottom ring. The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer.

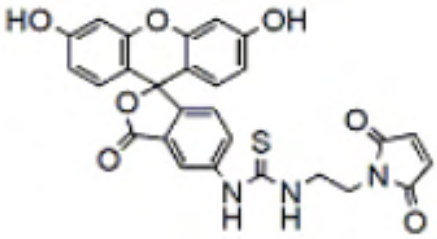
- Amine and thiol-specific labeling: the NHS-ester and maleimide efficiently labels antibodies and other purified proteins at primary amines or thiol.
- Optimized procedure: following the standard protocol results in antibodies with excellent dye:protein ratios for optimum activity and fluorescence
- Single-use fluors: no need to weigh tiny amounts powder; kits contain single-use vials of reagent
- Efficient purification: kits include purification resin and easy-to-use spin columns, ensuring rapid and efficient removal of non-reacted dye and excellent protein recovery.

Image

Dye	Laser channel	Filter	Abs max	Emi. max	extinction coefficient	CF_{280}
Fluorescein NHS	488	Cy5	494	518	75,800	0.35
Fluorescein Mal	488	Cy5	494	518	75,800	0.35







Protocol

Please follow general protocol
---dye maleimide antibody labeling kit
---dye maleimide antibody labeling kit

Application

Fluorescein is mainly used to label primary antibody and secondary antibody. The application:

- FC
- ICFC
- ICC
- IHC-F
- IHC-P
- IF
- IP
- WB

Reference

1. Dolina JS, Lee J, Griswold RQ, et al.; TLR9 Sensing of Self-DNA Controls Cell-Mediated Immunity to Listeria Infection via Rapid Conversion of Conventional CD4+ T Cells to Treg.; Cell Rep.; 1(31) 2020 107249
2. Daniel et al.; miR-9a mediates the role of Lethal giant larvae as an epithelial growth inhibitor in Drosophila.; 2018;Biology;
3. Bankoti et al. Differential regulation of Effector and Regulatory T cell function by Blimp1.; 2017; Scientific Reports;
4. Herr AE et al; On-chip coupling of isoelectric focusing and free solution electrophoresis for multidimensional separations, Anal Chem (2003) 75:1180-1187
5. Knight SC et al, Transfer of antigen between dendritic cells in the stimulation of primary T cell proliferation; Eur J Immunol (1998) 28:1636-1644
6. The TH, Feltkamp TE. Conjugation of fluorescein isothiocyanate to antibodies. II. A reproducible method. Immunology. 1970;18(6):875-881.

FAQ

Q: What biomolecules can be conjugated with the fluorescein NHS labeling Kit?
A: Proteins, peptides, and oligonucleotides can be conjugated with fluorescein. Proteins and peptides can be modified using primary amino groups on lysine residues and the N-terminus. Modified oligonucleotides with primary amino groups are suitable for conjugation.

Q: What determine the degree of fluorescein incorporation?
A: The degree of fluorescein incorporation depends on the concentration of protein, the molar excess of fluorescein added, the availability of primary amines in protein, and the incubation temperature and time of conjugation reaction.

Q: Are there other fluorescein dye for labeling available?
A: Yes, there are more than **26** fluorescein derivatives for customer's different needs including of acid, NHS, amine, alkyne, azide, hydrazide, DBCO.

Q: Is free fluorescein a concern following the conjugation protocol?

A: In immunoassays such as ELISA and Western blotting, the effect of free fluorescein is minimal almost of the unconjugated fluorescein is removed in the washing steps. However, free fluorescein must be removed in order to quantitate the amount of fluorescein incorporation.

Q: How can I determine the number of primary amines on my protein?
A: Without knowledge of the protein sequence, the number of primary amines can be estimated by multiplying the MW of the protein by 0.0006. This formula estimates the number of lysine residues given an average weight of 110 Daltons for an amino acid, and an average lysine content of 6.6% in a protein (Dayhoff, M.O., 1978). Due to steric or functional constraints, some lysine residues may not be available for conjugation.

Q: Unlabeled protein samples are stored in buffer with Tris, sodium azide, or glycine. Can I still conjugate the proteins to this Fluorescein NHS kit?
A: Tris, sodium azide and glycine will react with the NHS ester in the conjugation reaction. Remove them by buffer exchange against the conjugation buffer, then begin conjugation protocol

Q: Do I have to dissolve Fluorescein NHS in DMSO?
A: Fluorescein NHS is soluble in either DMSO or DMF. As with NHS esters exposure to aqueous buffers should be limited to prevent inactivation of Fluorescein NHS by hydrolysis.

Q: How long can you store rehydrated Fluorescein NHS?
A: It is not recommended that you store for future use. The 0.3 mg sizes of Fluorescein NHS is designed for single use, although multiple conjugates can be made.

Q: Why do I have to store Fluorescein NHS kit under desiccation?
A: The desiccation helps to remove moisture to prevent inactivation of NHS ester by hydrolysis. The Fluorescein NHS are packed in air-tight zip-lock bag with desiccants.

Q: My protein normally has an absorbance at 495nm. Can I still quantitate the amount of fluorescein?
A: If your unlabeled protein has an absorbance at 495nm, you may subtract the portion of the A495nm due to the unlabeled protein if you know the extinction coefficient at 495nm.

Q: My unlabeled protein contains a protein-based stabilizer such as BSA. How do I prepare my sample for conjugation?
A: BSA may be removed by commercially available albumin removal kits, such as Bio-Rad Affi-gel blue. If the unlabeled protein and contaminating protein are sufficiently different in size, an appropriate gel filtration column can be used to purify the sample. The contaminating protein can also be removed by immunoprecipitation, if you have an antibody to recognizethe contaminating protein.

Q: Adding more Fluorescein NHS brings the percentage of DMSO in the conjugation reaction above 5%. Can I still use this molar ratio of Fluorescein NHS?
A: Yes. Instead of dissolving Fluorescein NHS in DMSO at 4mg/mL, dissolve it at higher concentration in order to bring the percentage of DMSO in the final reaction to less than 5%.

Q: Do I have to purify my sample using the spin filters included in the Fluorescein NHS Kits?
A: No, you do not have to use the included 10,000 MWCO spin filters. The excess dye can be removed with a variety of desalting columns or dialysis cassettes. For conjugation to antibodies, 50,000 MWCO Vivaspin 6 centrifugal concentrators also work well.

Q: What is the extinction coefficient of Fluorescein NHS?
A: The absorbance of fluorescein is sensitive to pH. The variation of extinction coefficient depends on the pH of buffer used in absorbance measurement (conjugation buffer: borate buffer, pH 8.4, carbonate/bicarbonate buffer, pH 9.6). The extinction coefficient of FITC at pH is 81,000-85,000. At pH 7.8 the absorbance of FITC conjugates decreases by 8% (Bioconjugate Techniques, Greg T. Hermanson)

Q; How to calculate the D/P ratio?
A: Formula

$$\frac{\boldsymbol{Dye}}{\boldsymbol{Ab}} = \frac{\boldsymbol{A_{dye}} \times \boldsymbol{\varepsilon_{Ab}}}{(\boldsymbol{A_{Ab}} - \boldsymbol{CF} \times \boldsymbol{A_{dye}}) \times \varepsilon_{\boldsymbol{dye}}}$$

A_{dye} : dye fluorescein at 495nm absorbance
A_{Ab}: antibody at 280nm absorbance
ε_{Ab}, ε_{dye}: extinction coefficient of the antibody and 280nm and dye fluorescein at 495nm
CF: correction factor: 0.35

Q: Some reaction examples about relationship about D/P and amount dye added
A:

Protein concentration	Amount added	D/P
2mg/ml	50	16.1
	20	8.8
	10	4.8
	5	2.5
0.5mg/ml	50	15
	20	7.0
	10	4.7
	5	2.5
0.1mg/ml	50	4.5
	20	2
	10	1.3
	5	0.9

Q: What is ideal D/P ratio for fluorescein conjugates?
A: There is an optimum molar ratio of fluorophore to protein for the performance of Fluorescein -labeled protein conjugates in immunoassays. The ideal F/P ratio for a given Fluorescein -labeled conjugate will be dependent on the number and location of modified lysine groups in a particular protein. Different antibodies may show different behavior based on the locations of lysine groups in the antibody.

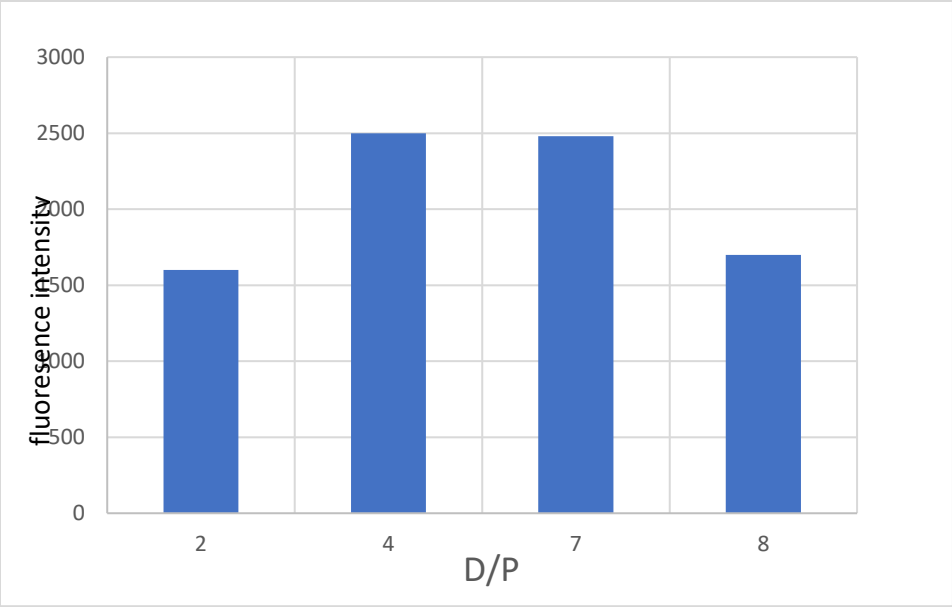


Figure 1 shows an example for goat anti-mouse IgG antibody conjugated to various amounts of Fluorescein dye and tested in an ELISA. In this experiment, the fluorescence intensity of the antibody- Fluorescein conjugate reaches a maximum at an F/P (fluorophore-to-protein) ratio of 4-7. Once the molar excess of Fluorescein goes beyond a certain point, the resulting fluorescent protein conjugates have a lower intensity, possibly due to steric hindrance or quenching between proximal fluorescein moieties.

Other Related Format Available

Reaction type		**Part number**
Click chemistry 1	Dye Azide	BP-22544, BP-22545, BP-20956, BP-23405,
	R-PEG- alkyne	See alkyne- PEG- NHS, alkyne- PEG- mal,
Click chemistry 2	alkyne	BP-22530, BP-22531,BP-23399
	R-PEG- azide	See azide - PEG- NHS, azide- PEG- mal,

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