HOOK™ Fluorescent Dye NHS Esters

Fluorescent Dyes-NHS Esters for Labeling Antibodies & Other Proteins

(Cat. # 786-1234, 786-1235, 786-1236, 786-1237, 786-1238, 786-1239, 786-1228, 786-1229, 786-1230, 786-1240, 786-1241, 786-1242, 786-1231, 786-1232, 786-1233)

INTRODUCTION

HOOK™ Fluorescent Dye-NHS Esters are range of fluorescent dye-NHS esters that are amine reactive and used for used for labeling antibodies, proteins, nucleic acids via amine group for application is biochemical detection assays.

All peptides and proteins have at least one primary amino group at the N-terminus of the peptide and several very reactive ε-amino groups from lysine residues depending upon protein sequence. For coupling of the Dye-NHS esters to nucleic acids, amino group is incorporated in such a way that the functionality and activity of nucleic acid is not altered, For example, 5'-aminomodified DNA oligomers.

HOOK™ Fluorescent Dye-NHS Esters react with non-protonated aliphatic amine groups that includes N-terminus amino group and ε-amino groups from lysine residues. Since the pK_a value for ε-amino groups from lysine residues is around 10.5, to maintain the amine group in non-protonated form, the conjugation reaction is carried in a buffer that has slightly basic pH. If specific labeling of only N-terminus amine group is desired then the conjugation buffer can be close to neutral pH as pK_a of terminal amine is lower than ε-amino groups from lysine.

ITEMS SUPPLIED

Cat. #	Description	Size
786-1234	HOOK™ 550 Dye-NHS Ester	1 mg
786-1235		5 mg
786-1236		10 mg
786-1237	HOOK™ 590 Dye-NHS Ester	1 mg
786-1238		5 mg
786-1239		10 mg
786-1228	HOOK™ 645 Dye-NHS Ester	1 mg
786-1229		5 mg
786-1230		10 mg
786-1240	HOOK™ 678 Dye-NHS Ester	1 mg
786-1241		5 mg
786-1242		10 mg
786-1231	HOOK™ 770 Dye-NHS Ester	1 mg
786-1232		5 mg
786-1233		10 mg

STORAGE CONDITIONS

Upon arrival store HOOK™ Dye-NHS Esters at -20°C. The HOOK™ Dye-NHS Ester when stored properly is stable for at least 4 months.

ADDITIONAL ITEMS NEEDED

- G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023) or SpinOUT™ GT-600 (Cat. # 786-170).
- PBS or desired buffer for conjugate purification.

FEATURES

- Dyes belonging to class of tri-, penta-, and hepta methane cyanines.
- Dyes have high molar extinction co-efficient.
- HOOK™ Dye NHS esters bind protein through their amine groups.
- HOOK™ Dye NHS esters are soluble in water, methanol, DMSO and DMF.

SPECIFICATIONS

Table 1: Fluorescent Dyes comparable to Alexa Fluor dyes

HOOK™ Dye-NHS Ester	**EX/EM maxima (nm)***	**Fluorescent color**	**Spectrally comparable Alexa Fluor dyes ****
HOOK™ 550 Dye-NHS Ester	553/568	Orange	Alexa Fluor 546, Cy3
HOOK™ 590 Dye-NHS Ester	584/598	Red	Alexa Fluor 568, Cy3.5, Texas Red, Promo Fluor 590
HOOK™ 645 Dye-NHS Ester	646/665	Red	Alexa Fluor 647, Cy5
HOOK™ 678 Dye-NHS Ester	677/703	Far Red	Alexa Fluor 660/680, Cy5.5, Promo Fluor 680
HOOK™ 770 Dye-NHS Ester	772/803	Far Red	Alexa Fluor 790

**Conjugation to an IgG antibody*

*** The fluorophores listed have similar excitation and emission properties to HOOK™ Dyes but may vary in brightness, photostability, water solubility, quantum yield, and pH response.*

Table 2: Properties of HOOK™ Dyes

HOOK™ Dye	λ_{max} (nm)	Em (nm)	ε_{dye}	CF_{280}
HOOK™ 550 Dye	553	568	150,000	0.08
HOOK™ 590 Dye	584	598	125,000	0.23
HOOK™ 645 Dye	646	665	250,000	0.05
HOOK™ 678 Dye	677	703	195,000	0.13
HOOK™ 770 Dye	772	803	200,000	0.07

λ_{max} = Fluorescence absorbance conjugated to IgG antibody

Em = Emission maxima conjugated to an IgG antibody

ε_{dye} = Extinction coefficient at λ_{max} in $cm^{-1}M^{-1}$

CF_{280} = Correction factor for absorbance readings (A280) at 280 nm.

PROCEDURE

Dye-NHS Ester reconstitution: Dissolve the amine reactive HOOK™ Dye-NHS Ester in DMF or DMSO at concentration 10 mg/ml.

NOTE: *Prepare only the required amount of HOOK™ Dye-NHS Ester solution in DMF or DMSO before conjugation reaction as its not very stable in this solution. Alternatively, the stock solutions can be prepared for the dye-ester in deionized water and stored at -20°C for later use.*

Antibody or protein Preparation

Dissolve or dilute the antibody or protein in 0.1 M sodium bicarbonate buffer, pH8.3 at concentration 10 mg/ ml.

NOTE: *The suitable concentration range of protein for labeling with fluorescent dye is 5-20 mg/ml*

NOTE: *The antibody must be dissolved in amine free buffer. If it is in amine containing buffer perform dialysis (G-Biosciences' Tube-O-Dialyzer™) or use desalting column (e.g.: G-Trap™ GT-600 Desalting Columns) to perform buffer exchange.*

Conjugation reaction

- Add 100 µl of 10 mg/ml of HOOK™ Dye-Ester to 1 ml of 10 mg/ml antibody solution.
  NOTE*: Optimization of dye-to-protein ratio may be necessary due to different reactivates of both the protein and fluorescent dye.*
- Close the lid of the vial and mix the solutions gently.
  NOTE: *Do not vortex.*
- Incubate the vial in dark at room temperature for 1 hour with intermittent gentle shaking every 15 minutes.

Isolation of conjugate

Conjugate can be isolated from unconjugated dye using either gel permeation chromatography (G-Trap™ GT-600 Desalting Columns*)* or spin columns (SpinOUT™ GT-600).

- Commonly used method is gel permeation. The column is equilibrated with PBS or desired buffer and elute using same buffer to collect the first colored band
- Alternatively spin column can be used to remove the unconjugated dye from conjugate.
- Dialysis and ultrafiltration can also be used though they are less efficient.

Calculation of degree of labeling

- Dilute a small amount of purified conjugate in PBS or suitable buffer to 0.1 - 0.3 mg/ml (absorbance at 280 nm in 0.15-0.5 range).
- Measure the absorbance of the diluted conjugate in a cuvette (1 cm path length) at 280 nm and at the absorbance maximum (λ_{max}) for the respective dye.
- Calculate molar concentration of protein in the diluted sample as below:

$$\text{Protein concentration (M)} = \frac{(A_{280} - A_{dye}) \times CF_{280}}{210{,}000}$$

NOTE: *210,000 is molar extinction coefficient (ε) in $cm^{-1}\,M^{-1}$ of IgG at 280 nm. This value is suitable for IgA, IgD and IgE as well. The value CF_{280} is a correction factor for the fluorophore contribution to the absorbance at 280 nm. It is determined by ratio A_{280}/A_{dye} for the free dye.*

NOTE: *Check table 2 for λ_{max} and CF_{280} values.*

The degree of labeling (DOL) is calculated as below:

$$\textbf{Moles of dye per moles of protein (DOL)} = \frac{A_{dye}}{\varepsilon_{dye} \times \text{Protein concentration (M)}}$$

NOTE: *ε_{dye} is the approximate molar extinction coefficient (in $cm^{-1}\,M^{-1}$) of the specific dye (check table 2 for ε_{dye} values).*

NOTE: For whole antibodies (MW: ~145- 150 kDa), acceptable degrees of labeling for the specific dyes are listed in table 3 below.

Table 3: Acceptable DOL for whole IgG

Dye	DOL
HOOK™ 550 Dye	2-4
HOOK™ 590 Dye	
HOOK™ 645 Dye	
HOOK™ 678 Dye	
HOOK™ 770 Dye	

Conjugate storage

Add sodium azide to the conjugate to a final concentration of 2 mM. Store in dark at 2-8°C. It can be stored like that for several months. For long term storage, divide the conjugate in smaller parts and store at -20°C.

NOTE: *Always protect from light and avoid freeze thawing.*

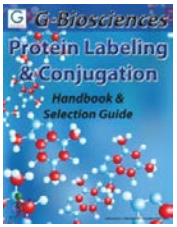
NOTE: *If the concentration of purified protein conjugate is less than 1 mg/ml, then add BSA or stabilizing agent at 1-10mg/ml concentration.*

TROUBLESHOOTING

Excessive-labeling	
Possible cause	**Possible solutions**
Degree of labeling is higher than expected	Increase antibody amounts
	Decrease the conjugation reaction time
	Add small amounts of dye solution to protein or antibody solution till the optimal degree of labeling is attained
Inadequate-labeling	
Possible cause	**Possible solutions**
Antibody buffer solutions contain primary amine contaminants	Dialyze or perform buffer exchange using column with desired buffer
pH of the conjugation solution is low	Add more bicarbonate buffer to get final pH value of 8.3
Different antibodies may react with reactive dye at different rates.	Optimize the labeling by changing conjugation reaction time or/and the amount of dye

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook



http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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