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A Geno Technology, Inc. (USA) brand name

HOOK™ Dye Labeling Kit

For Labeling Antibodies & Other Proteins with Fluorescent Dyes

(Cat. # 786-141, 786-142)

INTRODUCTION

FITC and *N*-Hydroxysuccinimide (NHS)-ester labeling reagents, such as (5/6)-TAMRA, are the simplest and most commonly used reagents for labeling proteins. The isothiocyanate group of FITC will cross-link with amino, sulfhydryl, imidazoyl, tyrosyl or carbonyl groups on a protein. However, only the derivatives of primary and secondary amines generally yield stable products. The (5/6)-TAMRA labeling reagent undergoes a cross-linking reaction between the NHS ester on the dye and primary amines on the protein that results in the formation of a stable, covalent amide bond.

The kit provides all reagents needed to perform 5 labeling reactions. The kit is provided with a dye reagent, a ready-to-use buffer for performing labeling reaction and Spin*OUT*™ columns to purify labeled antibodies and proteins.

ITEMS SUPPLIED

Cat. #	Description	Dye Labeling Buffer	Dye	Spin*OUT*™ GT-600
786-141	FITC Dye Labeling	5	5 x 1mg	5 columns
786-142	(5/6)-TAMRA-SE Dye Labeling	5	5 x 0.5mg	5 columns

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store at 4°C upon arrival. Stable for 1 year.

ADDITIONAL ITEMS NEEDED

Shaker, Stir plate and stir bar, DMSO or DMF

SPECIFICATIONS

Dye Labeling Agent Supplied	**FITC** *(Fluorescein Isothiocyanate)*	**(5/6)-TAMRA-SE** *(5(6) Carboxytetramethylrhodamine, succinimidyl ester)*
MW	389.5	527.5
Excitation/ Emission	494nm/ 520nm	558nm/ 575nm
Solvent	DMF/ DMSO	DMF/ DMSO
Molar ratio to protein	20	10 for >100kd protein, 5 for smaller protein

PREPARATION BEFORE USE

1. Dissolve one vial of Dye Labeling Buffer in 20ml deionized water. This buffer can be stored at 4°C and is stable for one week.
2. Warm the Dye Labeling Agent to room temperature before opening to prevent condensation and deterioration of the Dye Labeling Agent.

PROCEDURE

Protein Preparation

For optimal labeling, use 1mg protein (antibody) at approximately 2mg/ml concentration.

- For dry protein sample, dissolve 1mg of protein in 0.5ml Dye Labeling Buffer.
- For protein in PBS or Bicarbonate/Carbonate buffers, these samples are compatible with labeling reaction.
- For proteins in other solutions, the protein must be in a buffer free of amine (Tris and Glycine) and ammonium ions. Dialyze the sample against PBS. We recommend using our Tube-O-DIALYZER™ dialysis device to ensure no sample loss.

Dye Calculation

The amount of Dye Labeling Agent to use for each reaction depends on the amount of the protein to be labeled. The degree of labeling can be controlled by optimizing the ratio of Dye Labeling Agent to the protein. The guidelines for "optimal molar ratios" are provided in the Specifications section. They can be varied to alter the degree of labeling.

1. mmol protein = protein concentration (mg/ml) x protein volume (ml) / MW protein
2. mmol dye agent = mmol protein x molar ratio
3. µl dye agent = mmol dye agent x MW dye agent x 100 µl / mg dye agent

NOTE: Assume the dye-labeling agent is dissolved in 100µl solvent

For example: For labeling 500µl of 2mg/ml BSA (MW = 66340) with (5/6)-TAMRA-SE (MW = 527.5), dissolve 0.5mg (5/6)-TAMRA-SE in 100µl DMSO, you will need 1.6 µl (5/6)-TAMRA-SE solution:

1. mmol BSA = BSA concentration (mg/ml) x BSA volume (ml) / MW BSA

= 2mg/ml x 0.5ml / 66340

= 1.5x 10^{-5}mmol

2. mmol (5/6)-TAMRA-SE = mmol BSA x molar ratio

= 1.5x 10^{-5}mmol x 5

= 7.54x10^{-5} mmol

3. µl (5/6)-TAMRA-SE = mmol (5/6)-TAMRA-SE x MW (5/6)-TAMRA-SE x 100µl / mg (5/6)-TAMRA-SE

= 7.54x10^{-5}mmol x 527.5 x 100µl / 0.5mg

= 7.95µl Dye to add

Dye Preparation

1. Immediately before use, add 100µl of DMSO to one vial of Dye Labeling Agent. Pipette up and down until the agent is completely dissolved.

Labeling Reaction

1. Add the calculated volume of the *freshly prepared* Dye Labeling Agent solution to the protein solution. Quickly, vortex to mix then briefly centrifuge to collect sample in the bottom of the tube.
2. Wrap the tube with aluminum foil to protect from light. Incubate at room temperature for 60 minutes.

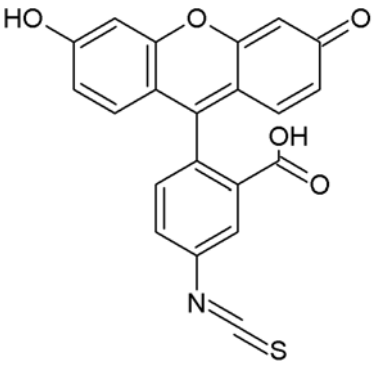
Removal of Unconjugated Dye

1. Mark one side of the Spin*OUT*™ GT-600 column and ensure in all centrifugations the mark is facing outwards during centrifugation.
2. Prepare the SpinOUT™ column by centrifuging the SpinOUT™ columns at 1,000g for 1 minute to compact the resin.
3. Remove the top and then bottom caps. Place into an appropriate collection tube.
4. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
5. Add 2ml Dye Labeling Buffer into to the column
6. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
7. Repeat steps 5 and 6, a further five more times, ensuring the buffer is discarded after each centrifugation.
8. Place the column in a new collection tube and remove the cap.
9. Slowly, apply the labeled protein solution to the center of the SpinOUT™ resin.
10. Centrifuge the column at 1,000g for 6 minutes to collect the protein solution. Discard the column.
11. Store the labeled protein at 4°C in 0.1% sodium azide for short-term storage. Freeze the labeled protein at -80°C and protect from light for long-term storage.

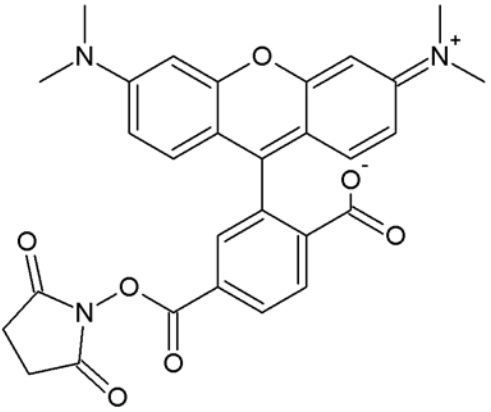
CITATIONS

1. Aktas, M. et al (2011) J. Bacteriol. 193:3473-3481

APPENDIX: CHEMICAL STRUCTURES



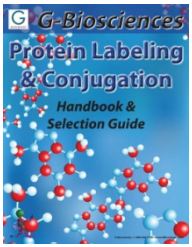
FITC
(Fluorescein Isothiocyanate)



(5/6)-TAMRA-SE
(5(6) Carboxytetramethylrhodamine, succinimidyl ester)

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook



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

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