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A Geno Technology, Inc. (USA) brand name

HOOK™-Dye Labeling Kit

For labeling antibodies & other proteins with fluorescent dyes

PROTOCOL SUMMARY

KIT COMPONENTS

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

STORAGE CONDITIONS

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

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.

ADDITIONAL ITEMS NEEDED

Shaker, Stir plate and stir bar, DMSO or DMF

SPECIFICATIONS

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

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 100 μl / 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. Invert the Spin*OUT*™ GT-600 column several times to re-suspend the gel material. Spin the column for 10 seconds at 1000x*g* to allow the gel to collect in the column. Do not exceed 1000x*g* as this will damage the resin.

2. Remove the tip of the column and let the liquid drain into a collection tube.

3. Spin-*OUT*™ GT-600 columns are supplied in deionized water containing a preservative, Equilibrate the column with 1ml Dye Labeling Buffer and let the buffer drain into the collection tube. Repeat this process 3 times, and discard the liquid collected in the collection tube.

4. Place the column in a 15ml centrifuge collection tube. Centrifuge at 1000xg for 2 minute, and discard the liquid collected in the centrifuge tube.

5. Place the column back in the same centrifuge tube. Carefully apply labeled protein sample to the center of the column without disturbing the resin bed. Wait for 1-2 minutes.

6. After loading the column, place the column in a new and clean collection tube and centrifuge at 1000x g for 4 minute. Collect the liquid containing purified sample.

7. Discard used column.

8. 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.

RELATED PRODUCTS

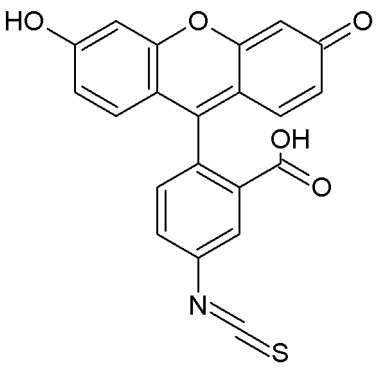
I. ***DMSO & DMF*** (Cat. # BKC-17 & BKC-16): *Vials containing anhydrous DMSO [Dimethyl sulfoxide $(CH_3)_2SO$] and DMF [N, N-Dimetylformamide HCON $(CH_3)_2$]. They are suitable for dye labeling and biotinylation reaction applications.*

II. ***Tube-O-Dialyzer***™ (Cat. # 786-610 to 786-624)*: Allows dialysis of small samples without taking the sample out of the tube and eliminates loss (Medi & Micro sizes available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).*

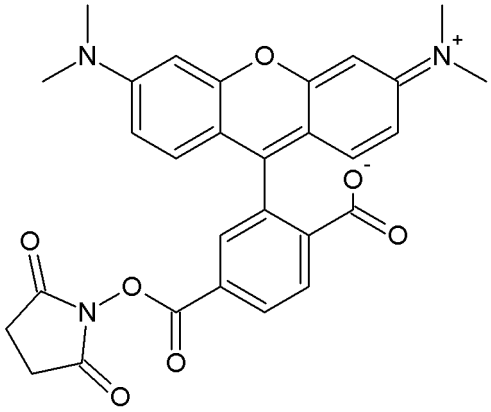
III. ***Spin-OUT™ GT-600*** (Cat# 786-170): *Equivalent to G-25 and is suitable for the purification of proteins > 6,000 molecular weight, and nucleic acids or oligonucleotides larger than 10bp.*

For additional related products, visit www.GBiosciences.com.

APPENDIX: CHEMICAL STRUCTURES



FITC *(Fluorescein Isothiocyanate)*



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

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