Marker Gene Technologies, Inc

Product Information Sheet

MarkerGene™ TAMRA Antibody/Protein/Cell Labeling Kit

Product M0972

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MGT

MarkerGene™ TAMRA Antibody/Protein/Cell Labeling Kit
(Product M0972)

NOTE: The following information is given as a viable methodology for use of the MarkerGene™ TAMRA Antibody/Protein/Cell Labeling Kit. Users may determine their own best methods for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

Direct labeling of a primary antibody eliminates the need to use a secondary antibody, providing lower background and higher sensitivity. The TAMRA (5(6)-carboxytetramethylrhodamine, succinimidyl ester) dye provided in this kit forms the desired dye-protein conjugates by reacting with non-protonated aliphatic amine groups, including the amine terminus of proteins and the ε-amino group of lysines. TAMRA is routinely used as a dye for carbohydrate and protein labeling and has even been used for direct labeling of live cells. The absorption and emission maxima of TAMRA conjugates are 555nm and 580nm, respectively. This kit can be used with a wide range of protein concentrations and with nearly all proteins having a MW of 25 kD or higher. TAMRA-antibody conjugates are useful in several applications including immunocytochemistry, flow cytometry, and fluorescence microscopy. The TAMRA Antibody/Protein/Cell Labeling Kit contains enough reagents for 5 protein labeling experiments or 25 cell labeling experiments, including buffers, solvents, gel-filtration chromatography media and a detailed protocol for use.

II. MATERIALS

A.) **1 TAMRA**: Five vials of 5(6)-carboxytetramethylrhodamine, succinimidyl ester reactive dye, each containing 500μg (#0972-001). 50μL of anhydrous DMSO is added to each vial, giving a reactive dye concentration of 10mg/mL.

B.) **2 Anhydrous Dimethylsulfoxide (DMSO)**: 0.7mL (#0972-002).

C.) **3 Reaction tubes**: 5 x 1.5 mL tubes (#0972-003).

D.) **Buffer Solutions**: **4** PBS Buffer: 1X concentration with 2mM sodium azide (#0972-004). **5** Sodium bicarbonate Buffer: 0.84g of $NaHCO_3$ is provided (#0972-005) and should be dissolved in 9mL deionized H_2O, adjusted to pH 8.3 with 2 N NaOH, and water added to give a final volume of 10mL.

E.) **6 Spin Column/Collection tube**: 2 spin columns containing 40-50mg Sephadex G-100 and 2 collection tubes (#0972-006).

F.) **7 Sephadex® G-100**: 250mg (#0972-007) of gel filtration resin is provided (fractionation range (MW) 4000-10000 for globular proteins, 1000-100000 for dextrans). 40-50mg should be added to a single spin column and equilibrated with provided PBS Buffer solution (or other buffer of choice). Based on the type of Sephadex® being used, one can separate different sizes of proteins or protein fragments or other molecules. For a better understanding, the table below describes the separation limits using various types of Sephadex®.

SEPHADEX® SEPARATION LIMITS (Biomedical Research, Clarendon Press, Oxford,1985)

SEPHADEX®	BEAD DIAMETER., μM	EXCLUSION LIMIT (M. WT)	EXCLUSION LIMIT (DALTONS)	BED VOLUME (ML/G DRY)
G-10	40-120	700	700	2-3
G-15	40-120	1500	1500	2.5-3.5
G-25	100-300	5000	5000	4-6
G-50	100-300	30,000	30,000	9-11
G-100	40-120	150,000	150,000	15-20

Storage and Handling: Protect reactive dye from light and moisture and store at –20°C . Reagents should be stable for at least 6 months following purchase.

Note: Two spin columns and two collection tubes are provided in this kit. Additional columns, collection tubes, and stir bars are not provided but may be required for dye-protein conjugate purification.

III. ANTIBODY/PROTEIN PREPARATION

This kit is designed for a maximum total reaction volume of 100μL, but the reaction can be scaled to accommodate other protein volumes. It is important to consider that the surface position and number of amines will vary among proteins and different IgGs, so it is recommended that different degrees of labeling be attempted, using different molar ratios of reactive dye to protein/antibody. Reactions that result in 2-4 TAMRA molecules per antibody molecule typically give optimum results. Modified protocols should be based on the amount of dye that gives the best results for your specific protein. Impure proteins will generally not label well.

Step 1: Dissolve purified protein in 1M $NaHCO_3$ buffer to give a concentration of 2-10mg/mL. Concentrations of less than 2mg/mL will decrease reaction efficiency. The sample should not contain any ammonium ions or primary amines (amine containing buffer salts, etc.). Antibodies (proteins) that are in unsuitable buffers, such as Tris or glycine, can be dialyzed against 10-20mM PBS and the desired pH for the reaction can be obtained by adding 100μL of 1M sodium bicarbonate buffer for each mL of antibody/protein solution. Presence of low concentrations of thimerosal (<1mM) or sodium azide (<3mM) will not affect the conjugation reaction. 200μL of this protein solution will be used in each labeling reaction.

IV. CALCULATIONS

This calculation should be determined before performing conjugation reaction. The amount of TAMRA dye needed for each reaction depends upon the protein concentration. There should be enough dye in each vial for two conjugations.

μL reactive dye solution =

$$\frac{\text{mg/mL protein} \times 0.1\text{mL}}{MW_{protein}} \times 527.53 \times 100 \times MR$$

(0.1mL is the volume of protein solution, 527.53 is the MW of the dye, 100 is a unit conversion factor, and MR is the molar ratio of dye to protein in the reaction. The following MRs are recommended: 1.) If protein or antibody is 2-3mg/mL use MR of 15 –20. 2.) If protein is 4-10mg/mL use MR of 8-10.

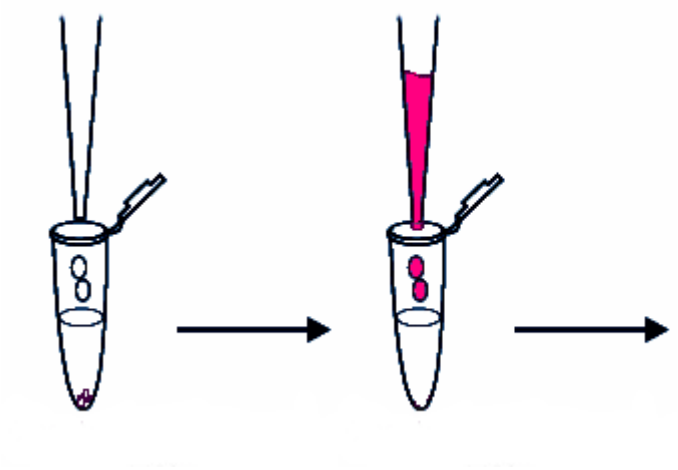
V. CONJUGATION AND PURIFICATION

Step2: Transfer 100μL of protein solution to a 1.5mL reaction tube[3]. Add 20μL of 1M $NaHCO_3$ buffer. After warming anhydrous DMSO[2] and a vial of reactive TAMRA dye[1] to room temperature, prepare 10mg/mL solution ***immediately*** before starting conjugation reaction, by adding 50μL of DMSO to one vial of TAMRA. Pipet contents up and down a few times to mix and dissolve. Add calculated amount of reactive dye solution to reaction tube, according to calculations above, and vortex immediately for 2-3 minutes. The remaining reactive dye solution can be discarded. The reaction is mixed thoroughly by brief vortex mixing, inversion, or by adding a small magnetic stir bar to reaction tube and stirring. Incubate at room temperature for 1-2 hours. If possible, keep reaction tube away from light. Total protein-dye solution volume should be in the range of ~120-150μL (100μL protein solution + 20μL 1M $NaHCO_3$ buffer + calc. volume of TAMRA dye solution).

Step 3: Prepare the spin column[6] by adding 40-50mg of Sephadex G-100[7] to empty column (if using column for the first time, gel is already supplied in column); gently tap the column to insure that the resin has settled to the bottom, keeping the column end stopper in place. Add 800μL of 1X PBS Buffer with 2mM sodium azide[4] replace top cap, and vortex for ~5 seconds. Tap bottom of column sharply to remove air bubbles. Allow at least 30 minutes of hydration time at room temperature before use. Once hydrated, first remove the top cap and then the column end stopper from the bottom, place in a 2mL collection tube, and centrifuge the column for 2 minutes at 1000 x g (maximum efficiency is obtained with swinging-bucket rotors but a fixed-angle-rotor will suffice). Discard buffer from collection tube. Do not allow column to dry excessively – process the labeled sample within a few minutes (if reaction volume exceeds 150μL, a single spin column will not adequately separate the conjugate from the free dye – the reaction should be divided and applied to multiple spin columns).

Step 4: Inspect the labeling reaction for any precipitate formation and centrifuge if necessary to remove the solid matter. Hold spin column up to the light and load sample or supernatant dropwise to the center of the column, without disturbing the gel surface. Allow solution to absorb into the gel bed with each drop. When finished, place the column in a collection tube and centrifuge for 5 minutes at 1000 x g, maintaining proper column orientation (the highest point of the gel media in the column should point towards the outside of the rotor). The purified sample, containing the labeled antibody (protein) in 1X PBS Buffer with 2mM sodium azide, will collect in the bottom of the collection tube. After collecting sample, throw away the used Sephadex G-100. The spin column can be washed 4-5 times with distilled water for reloading and reuse.

FIGURE 1. FAST AND SIMPLE METHOD FOR LABELING YOUR ANTIBODY.



Apply to
packed spin-column

With labeled protein

Step 1. Dissolve protein in 1M $NaHCO_3$, pH 8.3, to give concentration of 2-10mg/mL. Transfer 100µL to 1.5mL reaction tube. Add 20µL 1M $NaHCO_3$ buffer.

Step 2. Add calculated amount of reactive dye solution reaction tube containing protein solution. Vortex or mix stir bar at room temperature for 1-2 hours.

Step 3. Load sample dropwise to center of a packed column (see protocol for spin column preparation) and solution to absorb into gel bed with each drop.

Step 4. Place spin column in collection tube and centrifuge at 1000 x g for 5 minutes. The purified sample including labeled protein in 1X PBS buffer with 2mM NaN_3 will collect in bottom of tube.

VI. DETERMINING DEGREE OF LABELING

Read the absorbance of the TAMRA-protein conjugate at 280nm and 555nm*, using 1X PBS Buffer with 2mM sodium azide as the spectrophotometric blank and a path length of 1cm. Dilute the conjugate as necessary to obtain absorbance readings within the range of 0.2-1.5 OD. Calculate the protein concentration according to the Beer-Lambert law:

Absorbance = (**molar extinction coefficient**) x (**path length**) x (**concentration**)

$$(A=\varepsilon lc)$$

where ε is the extinction coefficient in $cm^{-1}M^{-1}$; path length is in cm; and the concentration is in moles/liter.

Concentration of protein:

$$\text{Protein concentration (M)} = \frac{Abs_{protein} \times \text{dilution factor}}{\varepsilon}$$

(ε is the molar extinction coefficient of the protein at 280nm. For most IgGs, $\varepsilon = 203{,}000 cm^{-1}M^{-1}$)
$Abs_{protein} = Abs_{280} - (0.30 \times Abs_{555})$ (0.30 is a correction factor to compensate for absorption of dye at 280nm, and Abs_{555} is absorption of the *dye* at 555nm.)

Concentration of TAMRA dye:

$$\text{TAMRA concentration (M)} = \frac{Abs_{555} \text{ dilution factor}}{65{,}000}$$

($65{,}000 cm^{-1}M^{-1}$ is the molar extinction coefficient of the TAMRA dye at 555nm)

Degree of Substitution (DOS): $\frac{\text{concentration of TAMRA}}{\text{concentration of protein}} = \textbf{DOS}$

***NOTE**: Absorbance readings may show a peak at 355nm due to self-quenching of the dye. This shouldn't affect degree of substitution calculations.

VII. LIVE CELL LABELING

For live cell labeling, cells are grown in 12-well tissue culture plates and incubated for 2 days at 37°C to allow growth to near confluent monolayers. Sterile coverslips can be added to some wells for photography purposes. Incubation of healthy cells with the reactive dye (50μg/well) can be accomplished by dissolving one vial of TAMRA [1] (500μg) in 2.5ml PBS buffer with sodium azide[4] plus 2.5ml of DMEM and pipetting 500μl of this solution per well to the live cells in culture. After 15 minutes at 37°C, the cells are washed with PBS and read (FACS, microscopy, etc.) with an excitation spectrum 460-565 nm, containing a peak at 550 and an emission spectrum 540-640 nm with a peak at 568. Consult your manufacturers technical information regarding appropriate filter sets or setup for photographic documentation or FACS analysis.

VIII. STORAGE OF CONJUGATES

Store labeled protein in PBS with 2mM sodium azide at 4°C, protected from light. Add BSA at 1-10mg/mL if protein conjugate concentration is less than 1mg/mL. Conjugate should be stable at 4°C for six months. Avoid repeated thawing and freezing.

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M0972 KIT CONTENTS			
DESCRIPTION	QUANTITY	PART NO.	STORAGE
REAGENTS			
1 TAMRA	5 x 500µg	0972-001	F, L, G
2 DMSO	0.7mL	0972-002	R, D, G
3 Reaction tubes	5 count	0972-003	N/A
4 PBS Buffer: 1X PBS buffer with 2mM sodium azide	1 x 2.5mL	0972-004	C, G
5 Sodium Bicarbonate (to prepare 1M buffer)	0.84 g	0972-005	C, G
6 Spin Column/Collection Tube	2 of each	0972-006	R, G
7 Sephadex G-100	250mg	0972-007	R, G
DOCUMENTATION			
MSDS Sheets	3		
Product Information Sheet	1		

Notes: F=store at or below -20°C; R=store at room temperature; C=store cold (4°C); L=light sensitive; D=store desiccated; FL=flammable; G=wear protective clothing/gloves/safety glasses when using; B=avoid breathing dust/fumes.

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