

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-LK-C002

Product size: 3 Reactions

Elabscience® CY3 Labeling Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Phone: 240-252-7368(USA) 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.

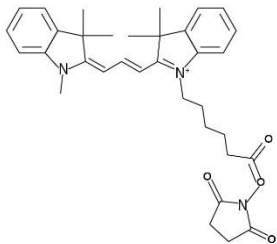
Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Introduction

The CY3 Labeling Kit of Elabscience offering a collection of reagents required for CY3 labeling is designed to label antibody with amidogen (NH₂-). The CY3 in this kit is sufficient and has been activated for direct use. And the reagents are enough for approximately 3 labeling reactions each containing 0.1-1 mg of antibody or other protein. Each kit includes 6 Filtration tubes for desalination of antibody labeling without the need for dialysis. The whole procedure is simple and can be completed in 100 min with proficient operation.

Information of the Reactive CY3

Chemical structure:



Product Features

- ✓ **All-inclusive:** This kit provides all the reagents required for CY3 labeling.
- ✓ **Quick:** The whole procedure takes only 100 min.
- ✓ **Convenient:** Desalination can be achieved with Filtration tube, dialysis or gel filtration is not necessary.
- ✓ **Flexible:** The procedure can be easily adapted to both micro and large scales, with 0.1-1mg of protein labeled each time.
- ✓ **Perfect results:** This kit has been optimized to determine the optimum labeling ratio of CY3 to antibody, lowering the possibility of protein inactivation resulted from insufficient labeling or excess CY3 labeling.

Product component and storage

The kit is stable for 1 year at 2-8 °C before opening.

The dissolved Reactive CY3 can be stored at 2-8 °C for 1 week.

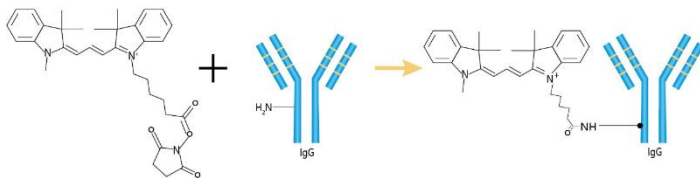
Component	3 Reactions
Reactive CY3	0.15 mg ×3 vials
Labeling Buffer	10 mL ×1 vial
DMF	500 μL ×1 vial
Filtration tube	0.5 mL ×6

Materials required but not included in this kit

1. Adjustable high-precision transferpeltor (10μL, 50μL, 200μL, 1000μL)
2. Incubator(37 °C)
3. Centrifuge(the centrifugal force can be up to 12,000×g)

Principle of the assay

The Reactive CY3 reacts with the primary amine (N-terminal and the side chain of lysine residue) specifically, forming stable amide bond.



Calculation on the amount of Reactive CY3

The volume of Reactive CY3 used in every reaction depends on the amount and concentration of the protein to be labeled. With optimization, we determine that the optimum molar ratio of the Reactive CY3 to protein is 20:1 when labeling 2 mg/mL of protein sample (IgG, 150KD).

1. Calculate the millimole of Reactive CY3 to make the ratio of CY3 to antibody is 20:1 when labeling 2 mg/mL antibody:

$$\begin{aligned} \text{mL protein} \times \frac{2\text{mg protein}}{\text{mL protein}} \times \frac{1\text{mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol Reactive CY3}}{\text{mmol protein}} \\ = \text{mmol Reactive CY3} \end{aligned}$$

2. Calculate the microliters of 10mM Reactive CY3 to add to the reaction:

$$\text{mmol Reactive CY3} \times \frac{1,000,000 \mu\text{L}}{\text{L}} \times \frac{\text{L}}{10 \text{ mmol}} = \mu\text{L Reactive CY3}$$

Example: About 13.3 μL of 10mM Reactive CY3 solution is to be added for 0.5 mL of 2mg/mL IgG(150,000 MW) solution.

$$\begin{aligned} 0.5\text{mL IgG} \times \frac{2\text{mg IgG}}{1\text{mL IgG}} \times \frac{1\text{mmol IgG}}{150,000\text{mg IgG}} \times \frac{20\text{mmol Reactive CY3}}{1\text{mmol IgG}} \\ = 0.000133\text{mmol Reactive CY3} \end{aligned}$$

$$\begin{aligned} 0.000133\text{mmol Reactive CY3} \times \frac{1,000,000\mu\text{L}}{\text{L}} \times \frac{\text{L}}{10\text{mmol}} \\ = 13.3\mu\text{L Reactive CY3 Solution} \end{aligned}$$

Preparation before experiment:

1. Read the manual carefully.
2. Calculate the volume of Reactive CY3 to be added.
3. Bring the kit to room temperature 20min before experiment (**Note:** the unused Reactive CY3 should be stored in the refrigerator).

Assay procedure (we label 1mg of protein in this assay)

1. Add 1mg of protein sample and corresponded volume of Labeling Buffer to a Filtration tube to make the volume is 0.5mL. Centrifuge at 12,000×g for 10min. **Note:** ①the maximum volume of Filtration is 0.5mL. ②the protein sample can be treated with centrifugal ultrafiltration first when at low concentration.
2. Dissolve Reactive CY3: add 20μL of DMF to the vial of Reactive CY3, let it stand for 10min until it dissolved fully. The concentration of Reactive CY3 is 10mM.
3. Add 13.3μL of Reactive CY3 and appropriate volume of Labeling Buffer to the Filtration tube, making the final concentration of the protein solution is 2mg/mL. Mix it thoroughly with a pipette and incubate the tube for 30min at 37 °C.
4. Centrifuge at 12,000×g for 10min.
5. Add appropriate volume of Labeling Buffer to the Filtration tube to make the total volume is 0.5mL. Mix it thoroughly with a pipette and centrifuge at 12,000×g for 10min. Repeat this step once again.
6. Add 0.2mL of Labeling Buffer to the Filtration tube and mix it thoroughly with a pipette. Invert the filtration tube and put it into another centrifugal tube. Centrifuge at 6,000×g for 10min.
7. Collect the solution in the centrifugal tube, namely antibody labeled by Reactive CY3.

Precautions

1. This kit can be also used to label antigen, HRP and polypeptides with amidogen (NH_2 -). The labeling ratio depends on the amount of amidogen.
2. DMF should be preserved airtight in a dry place. Seal it with the parafilm immediately after use.
3. The Filtration tube provided in the kit has a molecular weight cutoff(MWCO) of 10KD. So please be careful of the molecular weight of the antigen or polypeptide to be labeled.
4. In the Step 6 above, Labeling Buffer is used to collect the labeled protein. You can also use other buffer or protective agents as you like.
5. In the Step 3 above, for other quality antibodies, the final concentration of antibody should be controlled to 2 mg/ml strictly, then calculate the volume of Reactive CY3 required according to the quantity of the antibodies.
6. This kit can be stored for 1 year before opening. Please use it within the expiration date.