CY3 Labeling Kit
Catalog No: E-LK-C002
3 Reactions

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.

Phone: 240-252-7368(USA)    240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us 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 desaltination of antibody labeling without the need for dialysis. The whole procedure is simple and can be completed in 100 min with proficient operation.

**Product Features**

1. **All-inclusive:** This kit provides all the reagents required for CY3 labeling.
2. **Quick:** The whole procedure takes only 100 min.
3. **Convenient:** Desaltination can be achieved with Filtration tube, dialysis or gel filtration is not necessary.
4. **Flexible:** The procedure can be easily adapted to both smaller and larger scales, with 0.1-1mg of protein labeled each time.
5. **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**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive CY3</td>
<td>0.15 mg ×3 vials</td>
</tr>
<tr>
<td>Labeling Buffer</td>
<td>10 mL×1 vial</td>
</tr>
<tr>
<td>DMF</td>
<td>500 μL×1 vial</td>
</tr>
<tr>
<td>Filtration tube</td>
<td>0.5 mL×6</td>
</tr>
</tbody>
</table>

**Materials required but not included in this kit**

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

**Storage**

This kit can be stored at 2-8°C for 1 year before opening.
Principle
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 each reaction depends on the amount and concentration of the protein to be labeled. With optimization, we determine that the optimum molar ratio of the 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:

\[
\text{mmol CY3} = \frac{2 \text{ mg protein}}{\text{mL protein}} \times \frac{1 \text{ mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol CY3}}{\text{mmol protein}}
\]

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

\[
\text{μL CY3} = \text{mmol CY3} \times \frac{1,000,000 \text{ μL}}{L} \times \frac{L}{10 \text{ mmol}}
\]

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

\[
0.5 \text{ mL IgG} \times \frac{2 \text{ mg IgG}}{1 \text{ mL IgG}} \times \frac{1 \text{ mmol IgG}}{150,000 \text{ mg IgG}} \times \frac{20 \text{ mmol CY3}}{1 \text{ mmol IgG}} = 0.000133 \text{ mmol CY3}
\]

\[
0.000133 \text{ mmol CY3} \times \frac{1,000,000 \text{ μL}}{L} \times \frac{L}{10 \text{ mmol}} = 13.3 \text{ μL CY3 Solution}
\]
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 for 20 min before experiment (Note: The unused Reactive CY3 should be stored in the refrigerator).
4. Dissolve Reactive CY3: add 20 μL of DMF to the vial of Reactive CY3, let it stand for 10 min until it dissolved fully. The final concentration of CY3 is 10 mM

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

1. Add 1 mg of protein sample and corresponding volume of Labeling Buffer to a Filtration tube to make the total volume is 0.5 mL. Centrifuge at 12,000 ×g for 10 min.
   Note: ① The maximum volume of Filtration is 0.5 mL. ② The protein sample can be treated with centrifugal ultrafiltration first when at low concentration.
2. Add 13.3 μL of Reactive CY3 and appropriate volume of Labeling Buffer to the Filtration tube, making the final concentration of the protein solution is 2 mg/mL. Mix it thoroughly with a pipette and incubate the tube for 60 min at 37°C.
3. Centrifuge at 12,000×g for 10 min.
4. Add appropriate volume of Labeling Buffer to the Filtration tube to make the total volume is 0.5 mL. Mix it thoroughly with a pipette and centrifuge at 12,000×g for 10 min. Repeat this step once again.
5. Add 0.2 mL 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 10 min.
6. Collect the solution in the centrifugal tube, namely antibody labeled by CY3.
Precautions

1. This kit can be also used to label antigen, HRP and polypeptides with amidogen (NH₂-). 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. In the Step 5 above, Labeling Buffer is used to collect the labeled protein. You can also use other buffer or protective agents as you like.

4. This kit can be stored for 1 year before opening. Please use it within the expiration date.

5. The Filtration tube provided in this 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.

6. In the Step 2 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.

7. Ensure no free amino-group being introduced during the coupling process. (Tris, ammonia and sodium azide can react with the activated CY3, thus reducing the conjugation rate of protein-CY3).