Fluorescein One-Shot™ Antibody Labeling Kit

Cat. No.: F-9001-009K

Storage: 2° – 8°C — Do Not Freeze.

The Fluorescein One-Shot Antibody Labeling Kit requires 100 µg of antibody at a concentration of 1.0 mg/mL. The antibody buffer should be free of carrier proteins such as BSA or gelatin.

DESCRIPTION

The Fluorescein One-Shot Antibody Labeling Kit contains all the necessary reagents and components to label two 100 µg quantities of antibody with fluorescein. Based on SoluLINK® bioconjugation technology, it allows any antibody to be conjugated and purified within 90 minutes, involving just 30 minutes of hands-on time (Figure 1). The kit features high antibody recovery (70 – 90 µg) and a consistent level of fluorescein incorporation (3 – 5 fluorescein molecules per antibody) for reproducible results.

1. Start with 100 µl of antibody solution @1 mg/ml (0-5 min.)
2. Confirm antibody concentration (UV scan) (5 mins.)
3. Equilibrate spin columns & buffer exchange antibody (10 mins.)
4. Incubate antibody with NHS-Fluorescein (60 mins.)
5. Remove excess labeling reagent (2 mins.)
6. Quantify fluorescein incorporation (A280/A493) (8 mins.)

Figure 1. Fluorescein One-Shot Antibody Labeling Kit workflow.

PROTOCOL

A. Prepare antibody

Dilute the included 10X Modification Buffer to 1X concentration using ultrapure water. Use the 1X Modification Buffer to dissolve lyophilized antibody or dilute aqueous antibody solution to a concentration of 1.0 mg/mL. If the antibody is at less than 1.0 mg/mL, it must be concentrated prior to beginning. Centrifugal diafiltration apparatus are available which accommodate up to 500 µL of dilute antibody solution. Choose a molecular weight cutoff in the 10 – 30 kDa range and follow the manufacturer’s instructions for concentrating dilute protein samples.

B. Buffer exchange antibody

Once the antibody is confirmed to be at a concentration of 1.0 ± 0.1 mg/mL and a volume of 100 µL, buffer exchange the sample as follows:

1. Prepare two Zeba spin columns (red caps) by twisting off the bottom closures and loosening the caps one-half turn (do not remove completely). Place each spin column into a 2 mL collection tube.
2. Mark the top of one cap with the letter “A” and the other cap with the letter “B” using a lab marker.
3. Place a vertical mark on the side of each spin column using a lab marker.
4. Place each assembly into the centrifuge and orient the vertical mark on the spin columns facing outward (away from the center of the rotor).
5. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through from the bottom of the collection tubes, then place the columns back into the empty tubes. Important: Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.
6. Slowly add 300 µL of 1X Modification Buffer to the top of each column, then loosely re-cap.

Continued on next page.
7. Place each assembly into the centrifuge and orient the vertical marks facing outward.

8. Centrifuge at 1,500 x g for 1 minute. Discard the flow-through.

9. Repeat steps 6 through 8 two additional times.

10. Add 300 µL of 1X Modification Buffer to the top of column B, re-cap loosely, and set this column aside on the bench. It will be used later to buffer exchange the fluorescein-labeled antibody.

11. Transfer spin column A to a new 2 mL collection tube. Slowly add 100 µL of antibody at 1.0 mg/mL to the top of column A without disturbing the resin and loosely re-cap.

12. Place column A in the centrifuge and orient the vertical mark facing outward. Balance the rotor with a microcentrifuge tube containing water (do not use column B as a balance).

13. Centrifuge at 1,500 x g for 2 minutes.

14. Transfer the desalted antibody solution from the bottom of collection tube A to a labeled 1.5 mL microcentrifuge tube while measuring the volume with a P-200 pipet. Set the column A assembly aside to use as a balance later.

15. Measure the antibody concentration using a conventional UV-Vis spectrophotometer or a NanoDrop™ spectrophotometer to confirm antibody recovery.

16. If the antibody concentration is >0.8 mg/mL and >90 µL, proceed to section C.

**Important:** If the recovered antibody is below this volume and/or concentration, obtain additional antibody before proceeding.

**C. Label antibody with fluorescein**

1. Briefly centrifuge the vial containing NHS-Fluorescein at 10,000 x g to ensure the pellet is at the bottom. A very small yellow pellet should be visible.

2. Add 5.0 µL of anhydrous DMF to the pellet and pipet up and down until completely dissolved.

3. Add the desalted antibody solution directly to the vial of resuspended NHS-Fluorescein.

4. Immediately mix the solution by pipetting up and down several times, then gently vortexing.

5. Incubate the reaction for 60 minutes at room temperature in the dark.

**D. Buffer exchange fluorescein-labeled antibody**

1. Five minutes before the end of the 60-minute incubation period, place the equilibrated B spin column assembly (section B, step 10) containing 300 µL of 1X Modification Buffer into the centrifuge with the vertical mark facing outward.

2. Add 300 µL of water to the used spin column A assembly and place it in the centrifuge to serve as a balance.

3. Centrifuge the columns at 1,500 x g for 1 minute and discard the flow-through.

**Important:** Ensure the centrifuge is set to “g” or RCF rather than RPM in all centrifugation steps.

4. Transfer spin column B to a new collection tube.

5. Transfer the fluorescein-labeled antibody (section C, step 5) to the top of the column B resin and re-cap the column loosely.

6. Add 100 µL of water to column A and re-cap loosely. This column will serve as a balance.

7. Orient column B in the centrifuge with the vertical line facing outward and spin the columns at 1,500 x g for 2 minutes.

8. Transfer the fluorescein-labeled antibody from the bottom of collection tube B to a labeled 1.5 mL storage tube while measuring the volume with a P-200 pipet.

**Important:** Protect the fluorescein-labeled antibody from light.

**E. Measure the fluorescein MSR**

The fluorescein molar substitution ratio (MSR, or number of fluoresceins attached per antibody) is determined by measuring the sample using a conventional UV-Vis or NanoDrop spectrophotometer. Follow the instructions below for the type of instrument available.

**Conventional UV-Vis spectrophotometer MSR procedure**

1. Program the spectrophotometer to scan from 220 – 600 nm. If scanning is not available, measure the 280 nm and 494 nm absorbance values individually.

2. Using a clean semi-micro quartz cuvette (≤ 100 µL), blank the instrument using 1X Modification Buffer.

3. Discard the blank solution and dry the cuvette.

4. Transfer the labeled antibody sample to the cuvette and scan.

5. Record the 280 nm and 494 nm absorbance values from the scan.

**Important:** Recover the labeled antibody sample from the cuvette.
6. Enter the $A_{280}$ and $A_{494}$ values into the Fluorescein MSR Calculator, along with the antibody E1% value (Table 1), antibody molecular weight, and volume recovered. The calculator will display the fluorescein MSR and the fluorescein-corrected antibody concentration.

<table>
<thead>
<tr>
<th>Antibody Source</th>
<th>Antibody E1% (1-cm path)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human IgG</td>
<td>13.60</td>
</tr>
<tr>
<td>Human IgE</td>
<td>15.30</td>
</tr>
<tr>
<td>Rabbit IgG</td>
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<td>Goat IgG</td>
<td>13.60</td>
</tr>
<tr>
<td>Avian IgY</td>
<td>12.76</td>
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</tbody>
</table>

Table 1. Mass extinction coefficients (E1%, 280 nm) for antibodies derived from different host species. The E1% is used to calculate antibody concentration and represents the $A_{280}$ of a 10 mg/mL solution measured in a 1-cm pathlength cuvette. Most antibodies of the IgG isotype have a molecular weight of 150,000 Da.

Using the fluorescein IgG control to validate MSR measurements

The Fluorescein One-Shot Antibody Labeling Kit includes a fluorescein-labeled antibody control. This consists of lyophilized fluorescein-labeled bovine IgG at a known fluorescein molar substitution ratio. The control can be used to check the accuracy of a spectrophotometer, and to validate MSR measurements.

To use the fluorescein IgG control, add 100 µL of water and pipet the solution up and down for at least 1 minute to fully dissolve the antibody to 1.0 mg/mL. Centrifuge the vial for 30 seconds at 1,500 x g, then scan the sample in a conventional UV-Vis or NanoDrop spectrophotometer as described in section E. The fluorescein IgG control has an MSR value of 4.5 ± 1.5.

STORAGE

The fluorescein-labeled antibody should be stored at 2 – 8°C in the dark. A bacteriostatic agent such as 0.05% sodium azide or 0.01% thimerosal may be added to prevent microbial growth and extend shelf-life.

APPLICATION NOTES

Troubleshooting guide