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Protocol for Labeling IgG with Cyanine 3 maleimide [equivalent to Cy3® maleimide]

IMPORTANT DISCLAIMER: The following is a sample protocol for labeling targets with Cyanine 3 maleimide [equivalent to Cy3® maleimide]. This protocol only provides a guideline and should be modified according to your experimental needs. Please read the entire protocol before starting.

Additionally, this labeling protocol is not suitable for antibodies or proteins containing bovine albumin serum (BSA), gelatin, free amino acids or ammonium salts. Antibodies or proteins containing these impurities will have a very low conjugate yield, and as such require extensive purification. For such antibodies or proteins, AAT Bioquest offers custom bioconjugation services.

How to use this protocol:

First, select your protein from the dropdown menu below. If you selected 'Custom Protein' you will need to manually enter the molecular weight of your protein. Next, follow the instructions provided in each section to prepare your protein conjugates. For assistance, use the tools and calculators to determine the amount of component required for each part of the conjugation reaction.

Select protein

Protein:	IgG - Immunoglobulin G
Mol. Wt. (kDa?)	150

Prepare these materials

- Cyanine 3 maleimide [equivalent to Cy3® maleimide]
- IgG
- 1 M Sodium Bicarbonate Buffer
- 1 M Dithiothreitol (DTT)
- PBS, pH 7.2-7.4
- 1 M NaOH or 1M HCl
- DMSO
- Sephadex® G-25, Bio-Gel® P-6 DG Media or other desalting column
- Spin Column

1 M Sodium Bicarbonate Calculator

To calculate the mass of sodium bicarbonate needed to make a 1 M Sodium Bicarbonate buffer, enter your desired volume below. Note: $NaHCO_3$ molecular weight is 84.007 g/mol.

Desired Volume:	50	µL
$NaHCO_3$ Mass:	4.2	mg

To make1 M $NaHCO_3$ solution:

1. Add 4.2 mg of $NaHCO_3$ to a suitable container.
2. Then add distilled water until the volume is 50 µL.
3. Test the pH of your buffer, the pH should be between 8.5 to 9.5.
4. If the pH is below 8.5, adjust accordingly by adding 1 M NaOH.
5. If the pH is above 9.5, adjust accordingly by adding 1 M HCl.

Step-by-step guide

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1. Prepare a PBS buffer (pH 7.2 - 7.4) and a 1 M sodium bicarbonate solution.

1. For instructions on how to prepare PBS buffer (pH 7.2-7.4), see our buffer recipe page.
2. For instructions on how to prepare a 1 M sodium bicabonate solution use the above calculator.

2a. Check IgG solution for any impurities.

1. Does your protein contain any preservatives such as sodium azide or small molecule stabilizers?
   ☐ Yes ☑ No
2. Was your protein dissolved in TRIS or glycine buffer?
   ☐ Yes ☑ No

2b. Additional steps may be required:

1. No steps required. Proceed to Step 3a!

3a. Prepare IgG stock solution.

1. Select the form of your protein:
   ☐ Solid (lyophilized) ☑ Liquid (resuspended)

3b. Prepare your IgG solution from liquid (resuspended) form.

1. Initial Protein Concentration:
   10 mg/mL
2. Desired Protein Concentration:
   10 mg/mL
3. Desired Volume:
   100 µL
4. Transfer 100 µL of your initial 10 mg/mL protein solution into a suitable container. Next add 0.00 µL of PBS buffer (pH 7.2-7.4) to give a final volume of 100 µL. The concentration of your protein labeling solution is 10 mg/mL, which is equal to 0.0667 mM.

3c. Treat IgG solution with DTT to reduce disulfide bonds.

1. Enter the volume (in mL) of protein solution to reduce 0.1
2. To reduce 0.1 mL of your protein solution, prepare a 2.000 µL stock solution of 1 M DTT by disolving 0.308 mg of DTT in 2.000 µL of deionized water.
3. Add 2.000 µL of your 1 M DTT stock solution directly to the 0.1 mL of protein stock solution you want to reduce (the final concentration of DTT should be 20 mM).
4. Let the mixture react for 30 to 40 minutes at room temperature without any additional mixing
5. Purify your reduced protein solution.
   - For protein solutions > 1 mL use a Sephadex G25® gel column.
   - For protein solutions ≤ 1 mL use a PD-10 spin column to dialyze the reduced mAb into 50 mM MES pH 6.0 + 0.15M NaCl + 2 mM EDTA.
6. Store buffer exchanged SH-protein solution on ice until coupling in Step 7.

4. Prepare a 10 mg/mL Cyanine 3 maleimide [equivalent to Cy3® maleimide] labeling solution.

1. Enter the amount of label (in mg) you wish to use1
2. In a suitable container, dissolve 1 mg of Cyanine 3 maleimide [equivalent to Cy3® maleimide] in 100 µL of DMSO to make a labeling solution with a concentration of 10 mg/mL.

5a. Check the pH your IgG solution.

1. Test the pH of your protein solution. Is the pH between 8.5-9.5?
   ☐ Yes ☑ No

5b. Additional steps may be required:

1. Adjust the pH of your protein solution by adding 1 M sodium bicarbonate, pH 8.5-9.5, at a volume equal to 5% of the antibody solution.

6a. Determine the optimal label to protein (label to IgG) ratio.

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1. Enter the amount of IgG (in mg) you wish to label 1
2. Select the desired label to IgG ratio. 10 to 1
   For best results, try 5:1, 10:1, 15:1 or 20:1 label to protein labeling ratios.

6b. Before running the conjugation reaction, do a final check of the following:

1. Ensure that the amount of DMSO accounts for less than 10% of the total reaction volume.
2. Make sure that the Cyanine 3 maleimide [equivalent to Cy3® maleimide] labeling solution, protein solution and all required buffers are ready before starting the conjugation reaction.

7a. Run your label to IgG conjugation reaction.

1. Using a 10:1 molar ratio to label 1 mg of your protein, mix 5.78 µL of Cyanine 3 maleimide [equivalent to Cy3® maleimide] solution into a vial containing 100 µL of your protein solution with effective shaking.
2. Continuously rotate or shake your reaction mixture at room temperature (37 °C) for 1 hour.
3. Follow the instruction in **Step 8** to purify your protein conjugate.

8. Purify your protein conjugates using a 1 mL spin column.

1. Prepare Sephadex® G-25, BioGel® P-6 DG Media or other desalting column according to the manufacture instructions.
   Note: 1 mL spin columns are good for purification of 100 µL test conjugate.
2. Drain the solution (top dry) and do a buffer exchange using ~4 mL PBS buffer (pH 7.2-7.4).
3. Spin column at 2000X for 2 minutes, then exchange the 15 mL tube for a brand new one.
4. Load your reaction mixture (directly from **Step 7**) to the top of the desalting column.
5. Add 10 µL of PBS buffer (pH 7.2-7.4).
6. Spin column at 2000X for 5 minutes.
7. Collect elution and measure concentration using a spectrophotometer (e.g. NanoDrop).
8. Determine your protein conjugate's Degree of Labeling, see **Step 9**.

9. Characterize your protein conjugates using our Degree of Labeling (DOL) calculator. The degree of labeling (DOL) is a useful parameter for characterizing the average number of label molecules that have covalently bonded to your sample protein during the labeling reaction.It can be determined from the absorption spectrum of your labeled bioconjugate.The optimal DOL for most antibodies is recommended between 2 and 10 depending on the properties of the label and protein.For effective labeling, the degree of labeling should be controlled to have 4-10 moles of label to one mole of protein.Click here for the DOL Calculator

10. Conclusion

1. If you are not satisfied with the DOL of your protein conjugate, you can return to Step 6a and try a different labeling ratio.
2. If you are satisfied with the DOL of your protein conjugate, then dilute with PBS buffer (pH 7.2-7.4) and aliquote for multiple uses.
3. If you want to use your protein conjugates later, store according to storage specifications.

Additional Information

Label Specifications

Excitation:	555 nm
Emission:	565 nm
Molecular Weight:	866.92
Solvent:	DMSO

1 M NaOH Recipe

1. Prepare 2 mL of distilled water in a suitable container.
2. Slowly add 100 mg of NaOH to the solution with mixing.*
3. Add distilled water until volume is 2.5 mL.
4. Store solution in plastic container at room temperature.
   *This is an exothermic process, proper precautions and guidelines should be followed.

1 M HCl Recipe

1. Prepare 2 mL of distilled water in a suitable container.
2. Slowly add 91.15 mg of hydrochloric acid (HCl) to the solution with mixing.
3. Add 500 µL of distilled water to solution, for a final volume of 2.5 mL
4. Store solution in a plastic container at room temperature, or discard after using.

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Storage Conditions

- Upon receipt, Cyanine 3 maleimide [equivalent to Cy3® maleimide] should be stored at < - 15 °C, dessicated and protected from light.
- Cyanine 3 maleimide [equivalent to Cy3® maleimide] reconstituted DMSO stock solutions can be stored at < - 15 °C for less than two weeks.
- Protein conjugates should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin).
- The conjugate solution can be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and protected from light.
- For long-term storage, protein conjugates must be lyophilized or divided into single-used aliquots and stored at ≤ –60 °C, and protected from light.