iFluor® 800 maleimide

Catalog number: 1378
Unit size: 1 mg

Component	Storage	Amount (Cat No. 1378)
iFluor® 800 maleimide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

In vivo fluorescence imaging uses a sensitive camera to detect the fluorescence emission from fluorophores in whole-body living small animals. To overcome the photon attenuation in living tissue, fluorophores with long emission at the infrared (IR) region are generally preferred. Recent advances in imaging strategies and reporter techniques for *in vivo* fluorescence imaging include novel approaches to improve the specificity and affinity of the probes and to modulate and amplify the signal at target sites for enhanced sensitivity. Further emerging developments aim to achieve high-resolution, multimodality, and lifetime-based *in vivo* fluorescence imaging. Our iFluor® 800 is designed to label proteins and other biomolecules with infrared fluorescence. Conjugates prepared with iFluor® 800 have excitation and emission in the IR range. iFluor® 800 dye emission is well separated from commonly used far-red fluorophores such as Cy5, Cy7, or allophycocyanin (APC), facilitating multicolor analysis. This fluorophore is also useful for small animal in-vivo imaging applications or other imaging applications requiring IR detection. iFluor® 800 maleimide is thiol-reactive and can be readily used to conjugate thiol-containing biomolecules.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Prepare iFluor® 800 maleimide stock solution

1. Allow the vial of iFluor Dye maleimide to warm up to room temperature.
2. Add anhydrous DMSO to the vial to prepare a 10 mM dye stock solution.
3. Vortex the vial briefly to fully dissolve the dye, and then centrifuge to collect the dye at the bottom of the vial.
4. Protect all stock solutions from light as much as possible by wrapping containers in aluminum foil.

Prepare antibody or protein solution for labeling

1. If your protein already contains a thiol group, prepare the protein at 50-100 uM (for example: 5mg/ml BSA is ~75uM) in 50~100 mM MES buffer or buffers of your choice with pH 6.5~7.0.
2. If labeling with an intact antibody, reduction of disulfide bonds need to be carried out before maleimide reaction. Prepare antibody in 2-10 mg/ml in a suitable buffer with pH 7.0–7.5. A 10-fold molar excess of a reducing agent such as DTT or TCEP is added to the antibody. If DTT is used, it must be removed by dialysis or desalting to a suitable buffer with pH 6.5~7.0 prior to conjugation. If TCEP is used, it is not necessary to remove excess TCEP during conjugation with maleimides, however, removal of TCEP by dialysis or desalting prior to conjugation gives the better labeling efficiency.

   Below is a sample protocol for generating free thiol groups on antibody:

   1. Prepare 2-10mg/ml IgG solution in PBS.
   2. Prepare a fresh solution of 1 M DTT (15.4 mg/100 µL) in distilled water.
   3. Add 1- 20 µL of DTT stock per ml of IgG solution while mixing.
   4. Let the solution stand at room temperature for 30 minutes without additional mixing (to minimize the re-oxidation of cysteines to cystines).
   5. Pass the reduced IgG over a filtration column pre-equilibrated with 50 mM MES buffer (pH=6.5) to remove excess DTT.
   6. Determine the antibody concentrations. This can be done either spectrophotometrically or colorimetrically.
   7. Carry out the conjugation as soon as possible after this step.

   Note: For the best results, IgG solutions should be > 2 mg/mL.

   Note: The reduction can be carried out in almost any buffer from pH 7 to 7.5, e.g., MES, phosphate, or TRIS buffers.

   Note: Steps 5 can be replaced by dialysis.

3. If your protein doesn't have a free thiol group or disulfide bond to reduce, a thiolation modification need to be carried out before maleimide conjugation (for example: using 2-Iminothiolane or 2-IT) to introduce sulfhydryl (-SH) groups to the original amino groups on protein.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the labeling IgG with iFluor® Dye maleimide. Further optimization may be required for your specific proteins.

Note: Each protein requires a distinct dye/protein ratio, which also depends on the properties of dyes. Over-labeling of a protein could detrimentally affect its binding affinity while the protein conjugates of low dye/protein ratio give reduced sensitivity.

Run Conjugation Reaction

1. Use a 10~20:1 molar ratio of iFluor Dye Maleimide dye: IgG as the starting point. While stirring or vortexing the protein solution, add a volume of dye stock solution to result in a dye: protein molar ratio of 10-20. For example, for 5mg/ml IgG (~33 uM), you would add dye to a final concentration of 0.33-0.66 mM.

   Note: We recommend using a 10:1 molar ratio of dye to protein. If the ratio is too low or too high, determine the optimal dye/protein ratio at 5:1, 15:1, and 20:1, respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the Conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1. Purify the conjugate on a gel filtration column, such as a Sephadex G-25 column or equivalent matrix, or by extensive dialysis at 4°C in

an appropriate buffer.

Recommended AAT Desalting Columns:

Volume of Reaction	Catalog#
0.6-1.0mL	Cat#60504: PD-10 Column https://www.aatbio.com/products/readiuse-disposable-pd-10-desalting-column?unit=60504
~0.1mL	Cat#60500: Spin Column https://www.aatbio.com/products/readiuse-bio-gel-p-6-spin-column?unit=60500

Optional: Characterize the Desired Dye-Protein Conjugate

Determining the Degree of Substitution (DOS) is crucial in characterizing dye-labeled proteins. Lower DOS proteins tend to have weaker fluorescence, but higher DOS proteins may also have reduced fluorescence. For most antibodies, the optimal DOS is between 2 and 10, depending on the dye and protein properties. For effective labeling, the degree of substitution should be controlled to have 5-8 moles of iFluor® 800 maleimide to one mole of antibody. The following steps are used to determine the DOS of iFluor® 800 maleimide-labeled proteins:

1. Measure absorption— To measure the absorption spectrum of a dye-protein conjugate, the sample concentration should be kept between 1 and 10 μM (For example: IgG conjugate: 10uM is ~1.5mg/ml), depending on the dye's extinction coefficient.
2. Read OD (absorbance) at 280 nm and dye maximum absorption (λ max = 801 nm for iFluor® 800 dyes). For most spectrophotometers, the sample (from the column fractions) must be diluted with de-ionized water so that the OD values range from 0.1 to 0.9. The O.D. (absorbance) at 280 nm is the maximum absorption of protein, while 801 nm is the maximum absorption of iFluor® 800 maleimide. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.
3. Calculate DOS using our DOS calculator: https://www.aatbio.com/tools/degree-of-labeling-calculator

EXAMPLE DATA ANALYSIS AND FIGURES

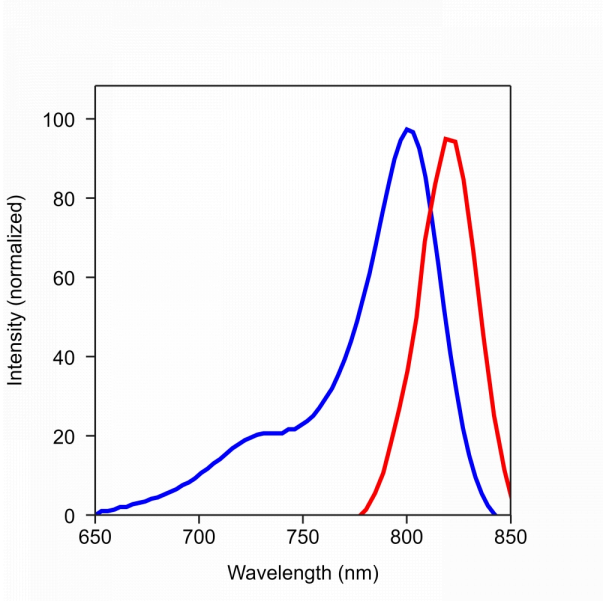

Figure 1. Spectrum for iFluor® 800

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