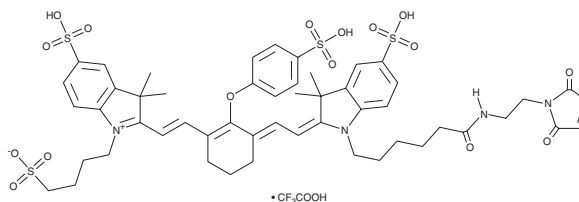


PRODUCT INFORMATION

iFluor™ IRD 800 CW Maleimide

Item No. 44615

MF: $C_{52}H_{60}N_4O_{16}S_4 \cdot CF_3COOH$
 FW: 1,239.3
 Purity: $\geq 90\%$
 Ex./Em. Max.: 774/789 nm
 Supplied as: A solid
 Storage: $-20^\circ C$
 Stability: ≥ 4 years



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

iFluor™ IRD 800 CW maleimide is a near-infrared thiol-reactive fluorescent dye. It displays excitation/emission maxima of 774/789 nm. iFluor™ IRD 800 CW maleimide can be used to label peptides, antibodies, and other thiol-containing biomolecules for use as probes in Western blot (WB), flow cytometry (FC), immunocytochemical (ICC) assays, and microscopy of cells, tissue sections, or tissues *in vivo*. iFluor™ IRD 800 CW maleimide penetrates greater than 1 cm into tissue with minimal scatter. Formulations containing iFluor™ IRD 800 CW-conjugated probes have been used as imaging agents in cancer diagnosis.

Assay Protocol

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

Prepare iFluor™ IRD 800 CW Maleimide Stock Solution

1. Allow the vial of iFluor™ IRD 800 CW maleimide to warm 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, 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 the protein-of-interest already contains a thiol group, prepare the protein at 50-100 μM (e.g. 5 mg/ml BSA is $\sim 75 \mu M$) in 50-100 mM MES buffer or a buffer of choice with pH 6.5-7.0.
2. If labeling with an intact antibody, disulfide bonds must be reduced before the maleimide reaction. Please see below for a sample protocol. In short, prepare the antibody at 2-10 mg/ml in a suitable buffer with pH 7.0-7.5. Add a 10-fold molar excess of a reducing agent, such as DTT or TCEP, to the antibody. If DTT is used, it must be removed by dialysis or desalting using 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 provides better labeling efficiency.
3. If the protein-of-interest does not contain a free thiol group or disulfide bond, a thiolation modification must be completed before maleimide conjugation (e.g. using 2-iminothiolane) to introduce sulfhydryl (-SH) groups to the original amino groups on the protein-of-interest.

WARNING

THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

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PRODUCT INFORMATION



Assay Protocol

Sample Protocol for Disulfide Bond Reduction in Antibodies

1. Prepare 2-10 mg/ml IgG solution in PBS.
2. Prepare a fresh stock solution of 1 M DTT (15.4 mg/100 μ l) in distilled water.
3. Add 1-20 μ l 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.

NOTE 1: For the best results, IgG solutions should be >2 mg/ml.

NOTE 2: The reduction can be carried out in almost any buffer from pH 7.0-7.5, e.g. MES, phosphate, or Tris buffers.

NOTE 3: Step 5 can be replaced by dialysis.

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: IgG as the starting point. While stirring or 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 μ M), 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.

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