# PRODUCT INFORMATION

## iFluor® 488 Maleimide

*Item No. 44062*

**Ex./Em. Max:** 491/516 nm
**Supplied as:** A solid
**Storage:** -20°C
**Stability:** ≥4 years
**Special Conditions:** Light sensitive

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

### Laboratory Procedures

iFluor® 488 maleimide is supplied as a solid. A stock solution may be made by dissolving the iFluor® 488 maleimide in the solvent of choice, which should be purged with an inert gas. iFluor® 488 maleimide is soluble in DMSO.

### Description

iFluor® 488 maleimide is a thiol-reactive fluorescent dye. It displays excitation/emission maxima of 491/516 nm, respectively, and exhibits pH-independent fluorescence from pH 4 to 10. iFluor® 488 maleimide can be used to label proteins and antibodies.

### Assay Protocol

*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® 488 Maleimide Stock Solution**

1. Allow the vial of iFluor® 488 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 ~75 µM) 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 or 2-IT) to introduce sulfhydryl (-SH) groups to the original amino groups on the protein-of-interest.



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## 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 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.

*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 of 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 a 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 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 5 mg/ml IgG (~33 µM), 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 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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## Optional: Characterize the Desierd 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® 488 maleimide maleimide to one mole of antibody. The following steps are used to determine the DOS of iFluor® 488 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: 10 uM is ~1.5 mg/ml), depending on the dye's extinction coecient.
2. Read OD (absorbance) at 280 nm and dye maximum absorption (λmax = 491 nm for iFluor® 488 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 491 nm is the maximum absorption of iFluor® 488 maleimide. To obtain accurate DOS, ensure the conjugate is free of the non-conjugated dye.
3. Calculate DOS



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