PROTOCOL

Antibody and protein near infrared (NIR) labeling with IVISense MAL Fluorescent dyes.

Introduction

Thiol (Sulhydryl) labeling agents, such as IVISense™ MAL Fluorescent Dyes, are commonly used for labeling proteins. The sulhydryl group reacts with maleimides at pH 6.5-7 to form stable thioether bonds. Thiols are very reactive functional groups in a protein and are available as targets for this chemical reaction.

Protein labeling efficiency may vary depending upon the type of protein labeled, so different conjugation ratios may need to be attempted to attain success. For Near Infrared (NIR) fluorophore conjugation, ratios of fluorephore to protein of 4:1 have been seen to generate effective imaging agents. Not all proteins (independent of target specificity) make good imaging agents, due to long half-lives and/or excessive accumulation in non-target sites.

General Protocol for Labeling a Thiol (Sulfhydryl) Containing Protein with IVISense MAL Fluorescent Dyes

Materials required

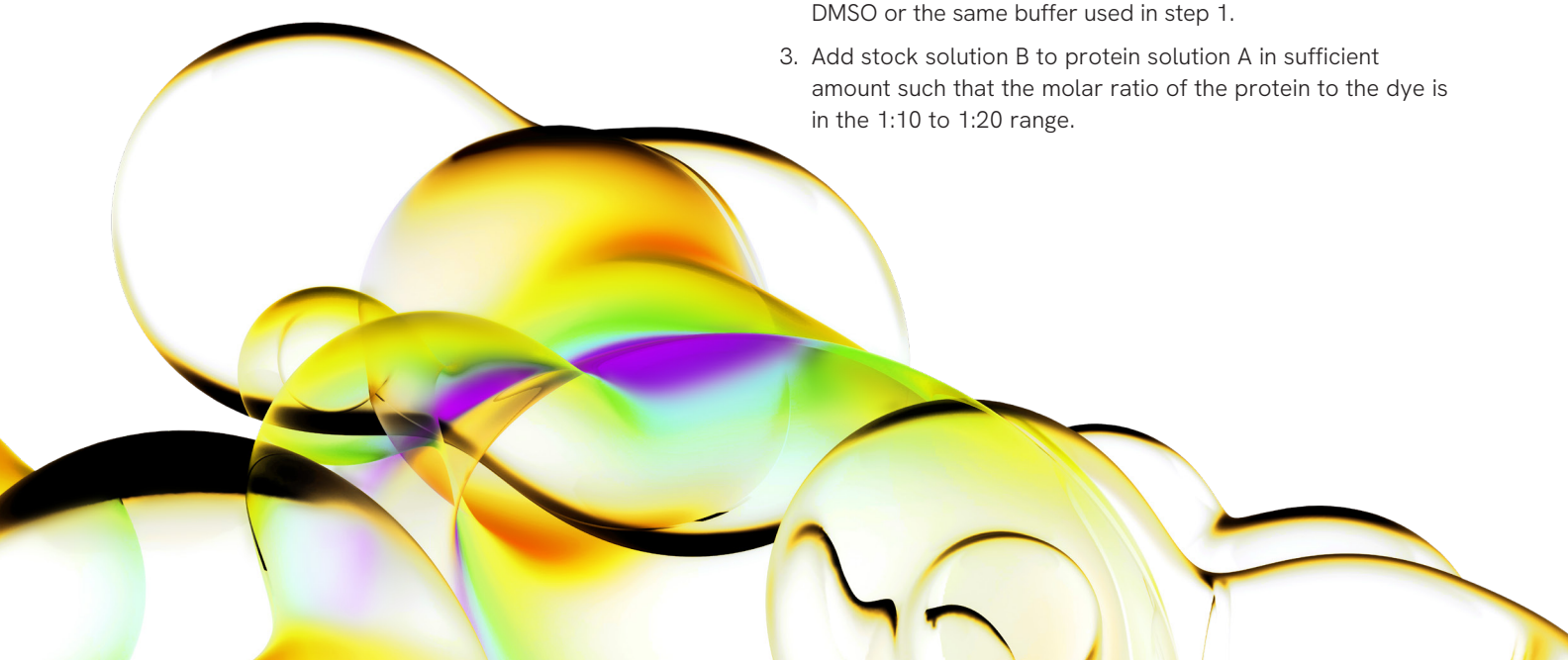
- Dimethylsulfoxide (DMSO)
- Conjugation Buffer*

1. Dissolve protein (A), which contains the thiol (sulfhydryl), in 0.25-1.0 mL of buffer at pH 6.5 – 7.0. *You may use any one of the following three buffers:

   100 mM PBS

   100 mM TRIS

   100 mM HEPES
2. Prepare a 10-20 mM IVISense Maleimide stock solution (B) in DMSO or the same buffer used in step 1.
3. Add stock solution B to protein solution A in sufficient amount such that the molar ratio of the protein to the dye is in the 1:10 to 1:20 range.



4. Wrap the reaction tube with aluminum foil and rotate at room temperature for 2 hours.
5. Next, add 5µL of mercaptoethanol and rotate at room temperature for 30 minutes in order to scavenge any excess IVISense Maleimide.
6. Separate the conjugated product by size exclusion chromatography (BioRad Bio-Gel P-100 or Sephadex G-25), or dialysis.
7. Sterile filter through a 0.2 µm syringe filter.
8. Store the labeled protein at 4°C in the dark until ready to analyze.

Table 1. Extinction coefficients.

IVISense MAL dye	ε (M^{-1} cm^{-1})	IgG
IVISense 645 MAL Epsilon	210,000	210,000
IVISense 680XL MAL Epsilon	210,000	210,000
IVISense 750 MAL Epsilon	240,000	210,000

Calculations of conjugation ratio

Take the wavelength absorbance readings of a 1: 10 dilution of your conjugate at the respective absorbance max of the dye (detects fluorophore):

643 nm for IVISense 645 MAL
670 nm for IVISense 680XL MAL
750 nm for IVISense 750 MAL

and at 280 nm (detects protein and some small contribution of fluorophore) blanked against PBS.

1. To accurately determine the correct A280 (i.e. adjust for fluorophore crosstalk), multiply the A643/A670/A750 value by the correction factor in the table 3, and subtract the resulting value from the A280. This gives you a more accurate protein absorbance (i.e. the abs due to protein only).
2. Calculate the protein concentration based on the extinction coefficient (in absorption units per Molar [M] concentration [A/M]) for your relevant protein, using the corrected A280. Convert protein concentration to M units.
3. Calculate fluorophore concentration using the extinction coefficient of your fluorophore.
4. The ratio of the fluorophore and protein molar concentrations will give the F:P ratio (Fluorescence to Protein ratio).

Table 2. Example calculations for IVISense MAL dyes.

	PBS	Ab	Comment
A280*DF	0	0.207	Absorbance at 280 nm
Corrected A280		0.1679	Corrects A280 based upon A750
A750*DF	0	0.782	Absorbance at 750 nm
Protein (M)		0.8x10-6	A280/210,000
Protein (mg/mL)		0.12	Convert M to mg/mL (IgG MW = 150,000)
Dye (M)		3.26x10-6	A750/240,000
Dye:Protein Ratio		4.1	Ratio of Dye to Protein values

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A. Pc = Conc. of protein = DF*[abs. at 280 nm – (abs. at 643/670/750 nm x Correction Factor*)] ÷ Epsilon of protein at 280 nm
B. Dc = Conc. of the IVISense MAL = abs. at 643/670/750 nm ÷ Epsilon of the IVISense MAL at 643/670/750 nm
C. Degree of labeling = Dc/Pc

*Use the corresponding correction factor as in Table 3

Table 3. Extinction coefficients.

Labeling dye	Correction factor
IVISense 645 MAL	0.05
IVISense 680 MAL	0.13
IVISense 750 MAL	0.05

Notes

Revvity's IVISense MAL dyes are intended for research purposes only and is not for human use. It must be used by or directly under the supervision of a technically qualified individual experienced in handling potentially hazardous materials. Please read the Material Safety Data Sheet (MSDS) provided for this product.

Several of Revvity's products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without specific agreement from Revvity.

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