Antibody and protein NIR labeling with IVISense NHS self-quenching dyes (NEV10121, NEV10122, NEV10123 & NEV10124).

Introduction

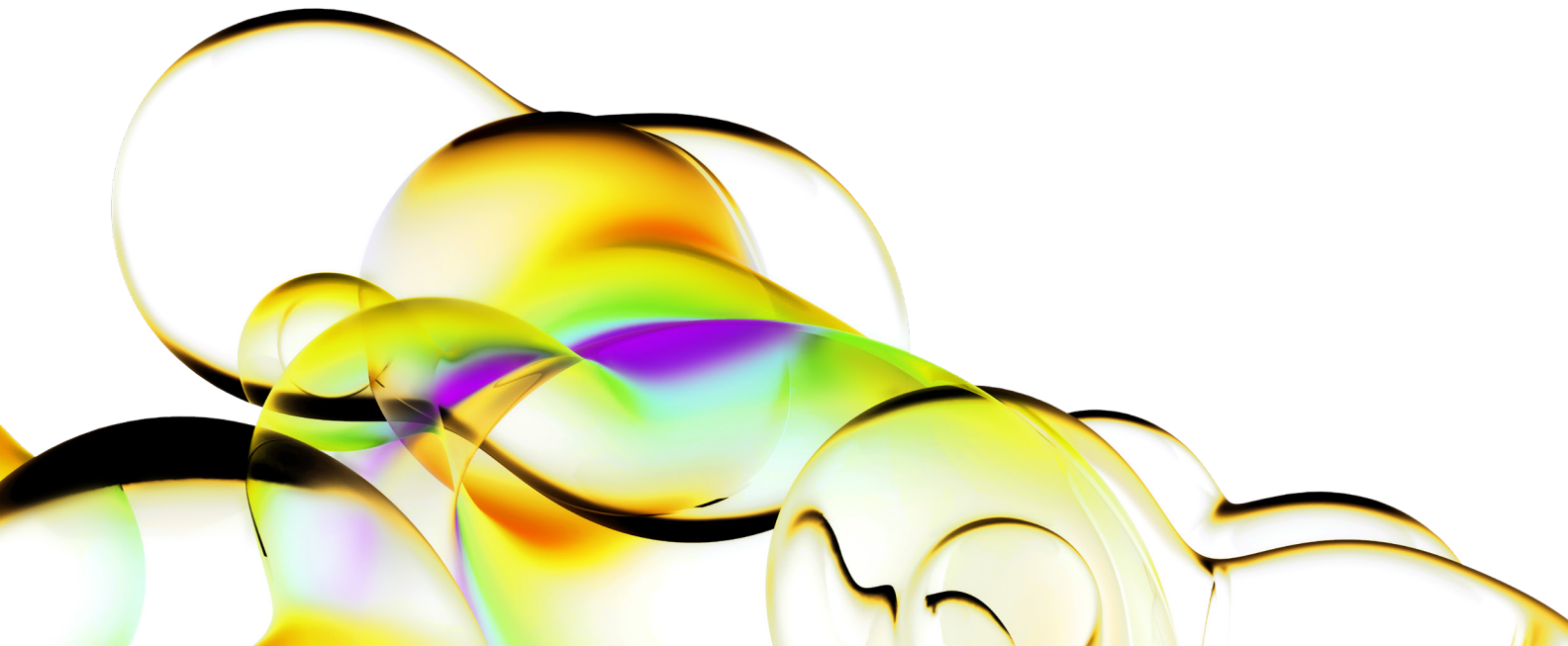
N-Hydroxysuccinimide (NHS)-ester labeling reagents, such as IVISense NHS self-quenching dyes, are commonly used for labeling proteins. The NHS ester moiety reacts with amino groups at pH 7-9 to form stable amide bonds. Lysines within proteins, including antibodies, are available as targets for this chemical conjugation.

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 fluorophore to protein of 4:1 have been seen to generate effective imaging agents. Not all antibodies or 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 an antibody with IVISense™ 680 or 750 NHS self-quenching dyes

Materials required

- Dimethylsulfoxide
- Conjugation Buffer:
  - 50 mM carbonate/bicarbonate buffer, pH 8.5



Steps

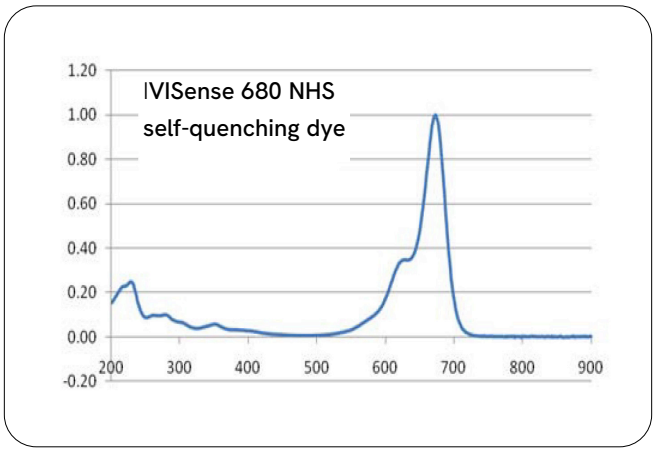
1. Prepare 1 mL of a 1 mg/mL solution of antibody in conjugation buffer.
2. Reconstitute 1 mg of IVISense NHS self-quenching dye with 100 µL DMSO.
3. Add 30 µL of IVISense NHS self-quenching dye to protein solution, mix well. **Note:** *it may be advisable to optimize conjugation amounts depending on the protein to be labeled.*
4. Incubate at room temperature for 1 hour.
5. Remove non-reacted fluorophore by size exclusion chromatography (BioRad, Bio-Gel P-100).
6. Sterile filter through a 0.2 µm syringe filter.
7. Store labeled protein at 4 °C in the dark until ready to analyze.

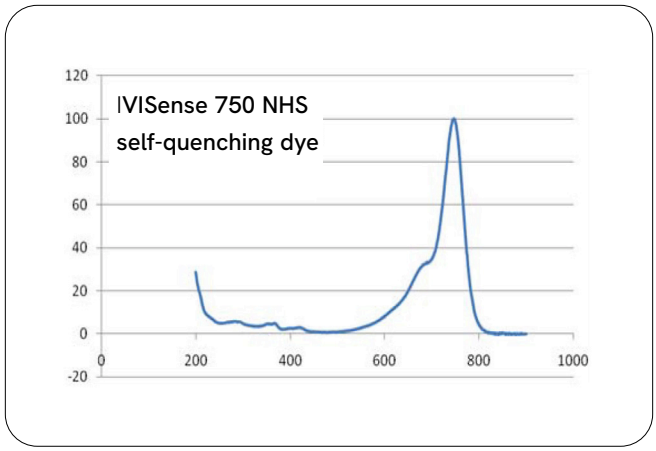
Extinction coefficients

	Dye	IgG
IVISense 680 NHS Epsilon	220,000 A/M	210,000 A/M
Absorbance 670 (A670) Crosstalk to A280	16%	
IVISense 750 NHS Epsilon	240,000 A/M	

Absorbance 280 is 6% of absorbance 750.

Optical absorbance/emission spectra of IVISense 680 and 750 NHS self-quenching dyes





Calculations of conjugation ratio

Take the wavelength absorbance readings of a 1:10 dilution of your conjugate at 670 nm (detects fluorophore) and 280 nm (detects protein and some small contribution of fluorophore) blanked against PBS.

1. Multiply A280 and A670 (or A750) results by the dilution factor (10).
2. To accurately determine the correct A280 (i.e. adjust for fluorophore crosstalk), multiply the A670/750 value by the percent of crosstalk for the appropriate fluorophore. Subtract this value from the A280. This gives you a more accurate protein absorbance (i.e. the portion due to protein absorbance only).
3. Calculate protein concentration based on the extinction coefficient (in absorption units per concentration in moles/liter [A/M]) for your relevant protein, using the corrected A280. Convert protein concentration to moles/liter units.
4. Calculate fluorophore concentration using the extinction coefficient of your fluorophore.
5. The ratio of the fluorophore and protein molar concentrations will give you the F:P ratio (fluorescence to protein ratio).

Example calculations for IVISense 680 and 750 NHS self-quenching dyes

Antibody conjugation

	PBS	Ab	Comment
A280	0	1.33	Absorbance at 280 nm
Corrected A280		1.03	Corrects A280 based upon A670
A670	0	1.84	Absorbance at 670 nm
Protein (moles/liter)	0	4.93×10^{-6}	A280/210,000
Dye (moles/liter)		0.84×10^{-5}	A670/220,000
Dye: Protein M ratio		1.7	Ratio of Dye to Protein moles/ liter values

Notes

Revvity's IVISense self-quenching dye is 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.

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