PROTOCOL

# Antibody and protein near infrared (NIR) labeling with IVISense 800 NHS dye (NEV11107 & NIEV11108).

## Introduction

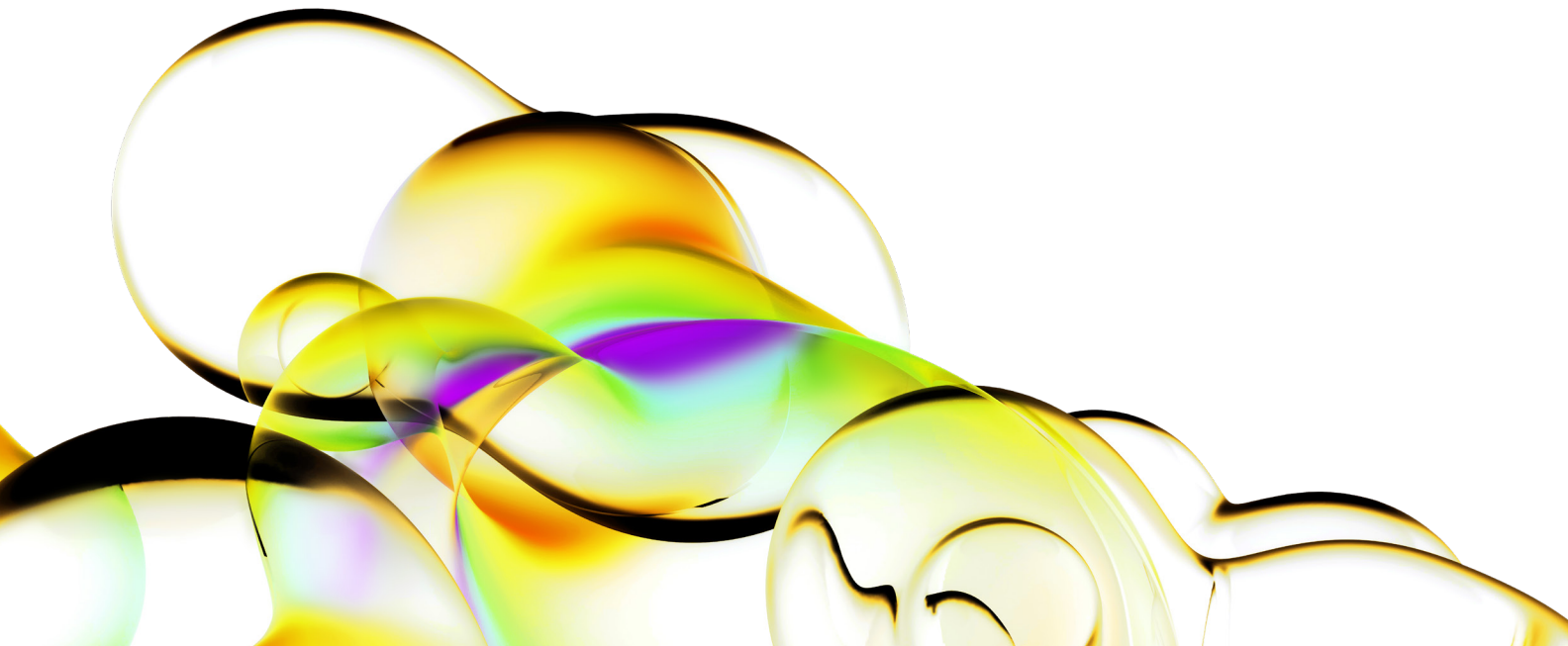
IVISense™ 800 NHS is a red fluorescent labeling dye containing an N-hydroxysuccinimide (NHS)-ester. Labeling reagents, such as IVISense 800 NHS reagent, is commonly used for labeling proteins. The NHS ester moiety reacts with amino groups at pH 7-9 to form stable amide bonds. Lysines within proteins, 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 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 800 NHS

**Materials required**

- Dimethylsulfoxide
- Conjugation Buffer:
  - Phosphate Buffered Saline (PBS), pH 7.4



## Steps:

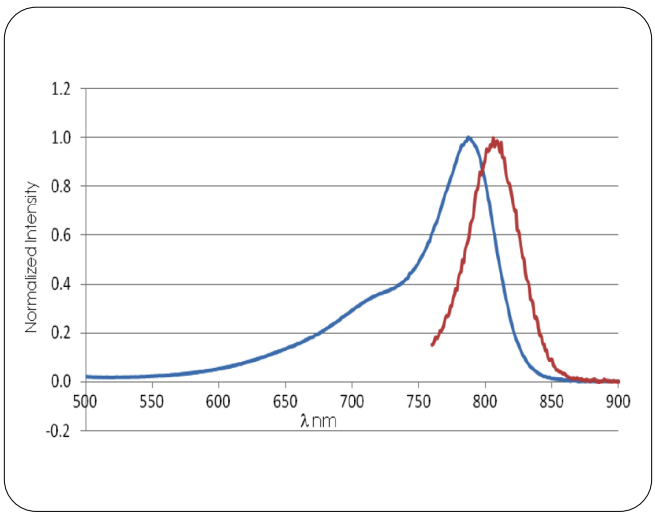
1. Prepare 1 mL of a 1 mg/mL solution of antibody in conjugation buffer.
2. Reconstitute 1 mg of IVISense 800 NHS with 100 µL DMSO.
3. Add 5-10 µL of IVISense 800 NHS dye to protein solution, mix well. **Note:** *it may be advisable to optimize conjugation amounts depending on the protein to be labeled.*
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 |
|---|---|---|
| Epsilon | 200,000 A/M | 210,000 A/M |

Absorbance 280 is 5% of absorbance 790.

## Optical absorbance/emission spectra of IVISense 800 NHS




## Calculations of conjugation ratio

Take the wavelength absorbance readings of a 1:30 dilution of your conjugate at 790 nm (detects fluorophore) and 280 nm (detects protein and some small contribution of fluorophore) blanked against PBS.

1. Multiply A280 and A790 results by the dilution factor (30).
2. To accurately determine the correct A280 (i.e. adjust for fluorophore crosstalk), multiply the A790 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 Molar [M] concentration [A/M]) for your relevant protein, using the corrected A280. Convert protein concentration to M 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 800 NHS

**Antibody conjugation**

| | PBS | Ab | Comment |
|---|---|---|---|
| A280 | 0 | 0.99 | Absorbance at 280 nm |
| Corrected A280 | | 0.833 | Corrects A280 based upon A790 |
| A790 | 0 | 3.15 | Absorbance at 790 nm |
| Protein (M) | | $3.96 \times 10^{-6}$ | A280/210,000 |
| Protein (mg/ mL) | | 0.595 | Convert M to mg/mL (IgG MW = 150,000) |
| Dye (M) | | $1.58 \times 10^{-5}$ | A790/200,000 |
| Dye: Protein M ratio | | 3.97 | Ratio of Dye to Protein M values |

## Notes

Revvity's IVISense NHS 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.

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