

Fluorescent Agents

PROTOCOL: Antibody and Protein Near Infrared (NIR) Labeling with VivoTag 800 Agents

Introduction

VivoTag® 800 is a red fluorescent labeling agent containing an N-hydroxysuccinimide (NHS)-ester. Labeling reagents, such as VivoTag 800 reagent, 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 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 VivoTag 800

Materials Required

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

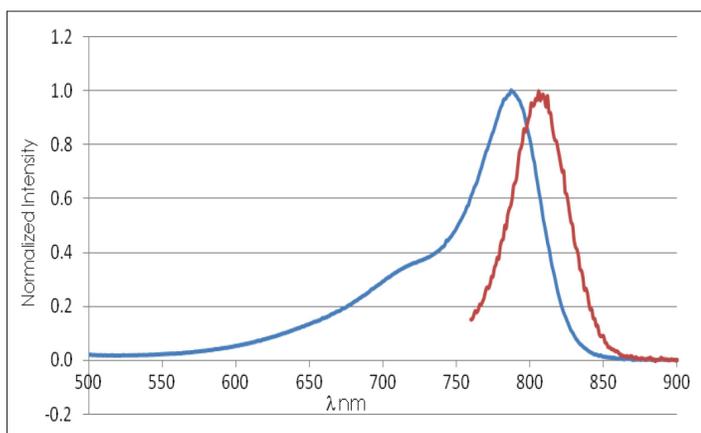
1. Prepare 1 mL of a 1 mg/mL solution of antibody in conjugation buffer.
2. Reconstitute 1 mg of VivoTag 800 with 100 µL DMSO.
3. Add 5-10 µL of VivoTag 800 to protein solution, mix well. **Note:** *it may be advisable to optimize conjugation amounts depending on the protein to be labeled.*
4. Incubate 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 VivoTag 800



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).

For laboratory use only. These products are intended for animal research only and not for use in humans.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

Example Calculations for VivoTag 800

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)		3.96×10^{-6}	A280/210,000
Protein (mg/mL)		0.595	Convert M to mg/mL (IgG MW = 150,000)
Dye (M)		1.58×10^{-5}	A790/200,000
Dye:Protein M ratio		3.97	Ratio of Dye to Protein M values

Notes

PerkinElmer's VivoTag 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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