

NIR Fluorochrome Labeling Kit

Caution: For Laboratory Use. A product for research purposes only.

VivoTag™ 680XL Protein Labeling Kit

Product Number: NEV11118

DESCRIPTION

PerkinElmer's VivoTag 680XL Protein Labeling Kit is designed for preparing fluorescently labeled antibodies, proteins or peptides for in vivo imaging applications in small animals. Each kit contains our superior in vivo optimized VivoTag 680XL fluorophore and everything else you need for carrying out the labeling reaction and purifying the labeled product.

The reactive fluorophore has a succinimidyl ester group, which reacts with an amine group on the protein (i.e., lysine side-chain amine) to form a stable amide linkage. VivoTag 680XL is supplied in two separate vials, each containing adequate material for labeling 0.5-5 mg of an antibody. Following the labeling reaction, the unconjugated fluorophore is conveniently and rapidly removed by purification columns with a molecular weight cutoff of 7 kDa

CONTENTS

- 2 X 0.25 mg of VivoTag 680XL
- 1 X 1 mL of 1M solution of sodium bicarbonate (pH8.3).
- 2 X Purification column
- 4 X 15 mL conical collection tube
- 1 XPBS (50 mL)

STORAGE & HANDLING

- Upon receipt, VivoTag 680XL Protein Labeling Kit should be **IMMEDIATELY STORED AT 2-8 °C AND PROTECTED FROM LIGHT.**
- When stored and handled properly, contents of the labeling kits are stable for up to six months.
- Allow VivoTag 680XL to equilibrate to room temperature before opening the vial.

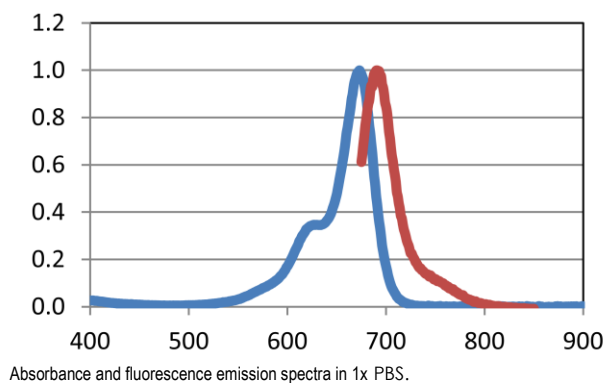
NOTES

- VivoTag 680XL Protein Labeling Kit 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.
- Several of PerkinElmer'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 a specific agreement from PerkinElmer.

PHYSICAL AND SPECTRAL PROPERTIES

Property	Specification
MW of VivoTag 680XL	1856 g mol ⁻¹
Fluorescence Emission max ¹	688 nm
Absorbance max ¹	668 nm
Extinction	210,000 M ⁻¹ cm ⁻¹
Purity ²	>95 %
Appearance	Blue solid

1. Absorbance and fluorescence maxima in 1x PBS.
2. As determined by SE-HPLC; measuring absorbance at 670 nm.



LABELING PROTOCOL

1. Prepare protein (>7 kDa) solution to 1-10 mg/mL in PBS. The protein must be free of ammonium ions or primary amines to reduce competition for reaction with the reactive dye.
2. Dissolve 0.25 mg of VivoTag 680XL in 10 μ L of dry DMSO. Once reconstituted, VivoTag 680XL is stable for up to 7 days when stored at 2-8 °C and protected from light.
3. In an Eppendorf tube, add 0.5 mL of protein (0.5-5 mg), 50 μ L sodium bicarbonate, 2 μ L of VivoTag 680XL for each mg of protein. Incubate in dark for 2 hours at room temperature with shaking.
4. Separate protein conjugate from free dye. Twist off the column's bottom closure and loosen cap. Place the column onto a 15 mL conical collection tube and centrifuge the column at 1,000xg for 2 min. Add 2 mL of PBS to the column and centrifuge the column at 1,000xg for 2 min. Repeat the wash two more times.
5. Place the column to a fresh 15 mL conical collection tube. Load all the protein samples (200-700 μ L) to the column and centrifuge at 1,000xg for 2 min. Collect the flow through protein sample.
6. The collected labeled antibody sample can be analyzed for the degree of labeling (DOL). Determine the absorbance of the purified conjugate at 280 nm and 668 nm.
7. Adjust the absorbance at 280 nm of the purified protein by subtracting the 280 nm absorbance of VivoTag 680XL, which is 16% of absorbance at 668 nm.
8. Absorbance analysis can be done with either a UV Spectrophotometer or a Nanodrop Spectrophotometer. To use the latter, samples need to be diluted to 0.5-2 mg/mL range before measurement. As the light path is 1 mm, the reading should be normalized with a factor of 10.

DEGREE OF LABELING CALCULATIONS

Protein concentration: (M) = $A_{280} - (0.16 \times A_{668} \text{ of dye}) / \epsilon$ (molar extinction coefficient). For antibody, ϵ is 210,000 $M^{-1}cm^{-1}$,

Dye concentration (M) = A_{668} / ϵ (molar extinction coefficient). ϵ is 210,000 $M^{-1}cm^{-1}$ for VivoTag 680XL.

DOL (Moles dye per mole protein) = Dye concentration (M)/Protein concentration (M).

Protein recovery should be close to 100%, DOL should be between 2 and 3.

PerkinElmer, Inc.
549 Albany Street
Boston, MA 02118 USA
P: (800) 762-4000 or (+1) 203-925-4602
www.perkinelmer.com/nenradiochemicals

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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