IVISense™ 680 NHS Fluorescent Labeling Kit

Product Number: NEV11118

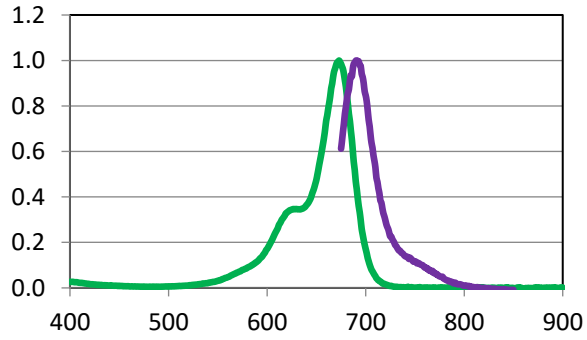
DESCRIPTION

IVISense™ 680 NHS Fluorescent 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 IVISense™ 680 NHS Fluorescent Dye 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. IVISense™ 680 NHS Fluorescent Dye 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.

Property	Specification
MW of IVISense™ 680 NHS Fluorescent Dye	1856 g mol^{-1}
Fluorescence[1] • Emission	688 nm
Absorbance[1]	668 nm
Extinction	210,000 $M^{-1}cm^{-1}$
Purity[2]	>95%
Appearance	Blue Solid

1. Absorbance and fluorescence in 1xPBS.
2. As determined by HPLC, measuring absorbance at 670nm



CONTENTS

- 2 X 0.25 mg of IVISense™ 680 NHS Fluorescent Dye
- 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, IVISense™ 680 NHS Fluorescent Labeling Kit should be IMMEDIATELY STORED AT 2-8 °C AND PROTECTED FROM LIGHT.
- For optimal storage conditions, refer to the labels on the individual components. Do not freeze the purification columns.
- When stored and handled properly, contents of the labeling kits are stable for at least three months.
- Allow IVISense™ 680 NHS Fluorescent Dye to equilibrate to room temperature before opening the vial.

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 IVISense™ 680 NHS Fluorescent Dye in 10 µL of dry DMSO. Once reconstituted, IVISense™ 680 NHS Fluorescent Dye 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 µL sodium bicarbonate, 2 µL of IVISense™ 680 NHS Fluorescent Dye 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 1,000x*g* for 2 min. Add 2 mL of PBS to the column and centrifuge the column at 1,000*xg* 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 µL) to the column and centrifuge at 1,000*xg* 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 IVISense™ 680 NHS Fluorescent Dye, 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}-(0.16xA_{668} of dye)/ ε (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 IVISense™ 680 NHS Fluorescent Dye.

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.

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