Oregon Green 488 Antibody Labeling Kit

O1491947

Storage For detailed storage information, please refer to the kit contents.

Introduction

The Oregon Green 488 Antibody Labeling Kit provides all necessary reagents for antibody labeling, including Oregon Green 488 NHS Ester, buffers, and a spin desalting column. The reactive dye efficiently conjugates to lysine residues in antibodies or proteins, forming a stable covalent product. The included spin purification column enables rapid removal of excess unreacted dye, with a recovery rate of 70%–95%. The kit contains 5 vials of reactive dye. Each vial is designed for labeling 100 μg of antibody.

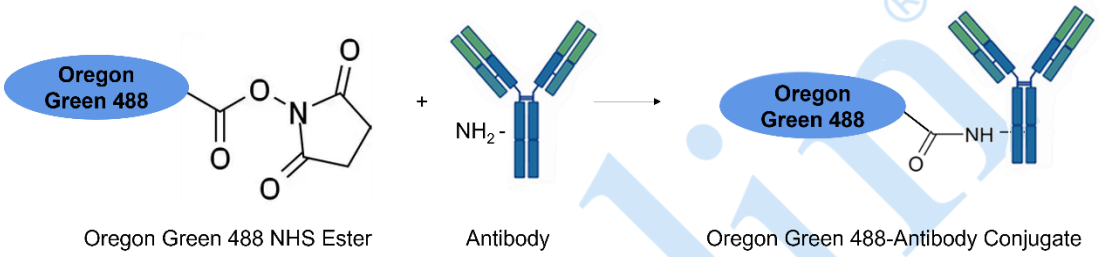

Fig. 1 Oregon Green 488 Antibody Labeling Kit (O1491947) Labeling Principle Schematic

Kit Contents

O1491947	Component	5 reactions	Storage	Quantity Per reaction
O1491947A	Oregon Green 488 NHS Ester	5 vials	-20℃. Store in the dark.	1 vial for labeling 100 μg of antibody
O1491947B	$NaHCO_3$	100 mg	RT.	Prepare according to instructions
O1491947C	Spin Desalting Column	5 EA	2-8℃. Do not freeze.	1 EA for 1 reaction
O1491947D	Collection Tubes	10 EA	RT.	2 EA for 1 reaction

Required materials not supplied

1. Microcentrifuge capable of 1,000 × g.
2. Desired antibody for labeling (free of BSA or any carrier protein).
3. PBS buffer (pH 7.2-7.4).

Matters needing attention

1. The purified antibody should be in a buffer that does not contain primary amines (for

example, ammonium ions, Tris, glycine, ethanolamine, triethylamine, glutathione) or imidazole. All of these substances significantly inhibit protein labeling.

2. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
3. Concentrate the antibody to ≥1 mg/mL.
4. Do not reuse the purification resin.
5. Before opening the vials, warm all components to room temperature.
6. The reactive dyes should be protected from prolonged exposure to light.

Instructions for Use

1. Prepare the reagents

(1) Prepare a 1 M sodium bicarbonate solution: Prepare an appropriate amount of 1M $NaHCO_3$ solution based on the sample volume. For example, weigh 42mg of $NaHCO_3$ and add 0.5mL of ultrapure water to obtain a 1M $NaHCO_3$ solution. $NaHCO_3$ can maintain the pH of the labeling reaction system between 7-9, thereby improving labeling efficiency.

Note: The $NaHCO_3$ solution must be prepared fresh before each use.

2. Label the antibody

(1) If the antibody to be labeled has a concentration of ≥1 mg/mL and is in an appropriate buffer, dilute it to 1 mg/mL, and add a 10% volume of 1 M sodium bicarbonate buffer. If the antibody is a powder lyophilized from an appropriate buffer, prepare a 1 mg/mL solution of the antibody by adding an appropriate amount of 0.1 M sodium bicarbonate buffer to the antibody. Prepare 0.1 M sodium bicarbonate buffer by diluting the 1 M solution 10-fold with ultrapure water.

(2) Transfer 100 µL of the antibody solution to the vial of reactive dye. Cap the vial and gently invert it a few times to fully dissolve the dye.

Note: To visually ensure that the dye has fully dissolved, peel the label off the vial of reactive dye.

(3) Incubate the solution for 60 minutes at room temperature in the dark. The reaction can be carried out on a shaker or mixer, recommended speed for flipping up and down is 25rpm. If a mixing instrument is not used during the reaction process, the reaction solution should be mixed upside down every 10 minutes.

Note: During the incubation period, proceed to steps 3 below, to prepare a spin column for the purification of the labeled protein.

3. Prepare the spin column

(1) Loosen the cap on a spin column, twist the tab off of the bottom, then place the column into a collection tube.

(2) Centrifuge the column-tube assembly at 1,000 × g for 2 minutes to remove the storage solution.

Note: When using a fixed-angle rotor, place a mark on the side of the column that faces away from the rotor center. For all subsequent centrifugation steps, place the column in the

microcentrifuge with the mark facing away from the rotor center.

(3) Discard the flowthrough, then place the column back into the collection tube.

(4) Add 500 µL of PBS Buffer, then centrifuge the column-tube assembly at 1,000 × g for 2 minutes to equilibrate the column. Repeat this step (3) 3 times, discarding the buffer from the collection tube each time.

4. Purification with Desalting Column

(1) Transfer the equilibrated column into a new collection tube.

(2) Carefully pipette the entire reaction mixture onto the center of the column.

(3) Centrifuge the column-tube assembly at 1,000 × g for 2 minutes. The purified antibody conjugate is in the collection tube.

5. Determine the antibody concentration and DOL (Optional)

(1) Use the absorption spectrophotometer to measure the absorption spectrum of the purified label, and record the absorbance values at 280 nm and 498 nm (with a 1-cm path length).

(2) The following formula can be used to calculate the antibody concentration:

Antibody concentration (mg/mL) = $(A_{280} - CF_{280} \times A_{498}) \times 150000/\varepsilon_{IgG}$

(3) The following formula can be used to calculate the degree of labeling:

DOL = $(A_{498}/ \varepsilon_{Oregon\ Green\ 488}) / [(A_{280} - CF_{280} \times A_{498}) / 210{,}000]$

Parameter	Meaning	Value
DOL	Represents the molar ratio of the fluorescent dye to the target molecule (e.g., antibody or protein) in the conjugate	-
A_{498}	Absorbance value of Oregon Green 488 at a wavelength of 498 nm	Measured value
$\varepsilon_{Oregon\ Green\ 488}$	Molar extinction coefficient of Oregon Green 488 ($M^{-1}cm^{-1}$)	76000
A_{280}	Absorbance value of the Oregon Green 488-labeled antibody at a wavelength of 280 nm	Measured value
CF_{280}	Correction factor for the absorbance value of Oregon Green 488 at 280 nm	0.12
ε_{IgG}	Molar extinction coefficient of the IgG antibody at 280 nm ($M^{-1}cm^{-1}$)	210000

6. Storage

(1) Add 0.05–0.2% Proclin 300 or 0.05% sodium azide, along with a protein stabilizer (such as 0.1% BSA), to the labeled protein. Store protected from light at 2–8°C for stable preservation up to six months. Alternatively, add an equal volume of glycerol and store at -20°C for stable preservation up to six months.