Oregon Green 488 Antibody Labeling Kit (1mg)

O1491970

Storage: Room Temperature. 2-8℃. -20℃. Protect from light. For detailed storage information, please refer to the kit contents.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

The Aladdin's Fluorescent Dye Antibody Labeling Kits are designed for efficiency and reliability, delivering ready-to-use labeled proteins in just 90 minutes, with minimal hands-on time. The kit contains all reagents required for antibody labeling, including amine-reactive dye, buffer, and purification columns. The reactive dyes contained in these kits readily react with lysine residues in antibodies or proteins to yield a covalently attached fluorescence-based sensor. Spin columns included in the kits are used for purifying the labeled antibody from excess dye with yields of 70–95%. The kit provides 5 vials of reactive dye, each containing an optimized amount of dye for labeling 1 mg of antibody.

We offer kits with a variety of dye colors, compatible with multiple detection systems, including fluorescence microscopy and flow cytometry.

For labeling smaller amounts of antibodies (~100 μg), we recommend our Fluorescent Dye antibody labeling kits (100μg).

Contents and storage

O1491970	Components	Appearance	5 Reactions	Storage	Quantity Per Test
O1491970A	Oregon Green 488 NHS Ester	Yellow solid	5 vials	-20℃. Store in the dark.	1 vial for labeling 1mg of antibody
O1491970B	DMSO	Colorless clear liquid	0.5 mL	RT.	Prepare according to instructions
O1491970C	$NaHCO_3$	White powder	500 mg	RT.	Prepare according to instructions
O1491970D	Purification Resin	White bead slurry	15 mL	2-8℃. Do not freeze.	3mL for 1 reaction
O1491970E	Empty Spin Column	Transparent tub	5 EA	RT.	1EA for 1 reaction

Number of labeling: Each vial of reactive dye contains the appropriate amount of dye to label approximately 1 mg of IgG (MW ~145,000) as 0.5 mL of IgG solution at 2 mg/mL.

Required materials not supplied

1. Centrifuge.
2. Antibody for labeling (without BSA or any carrier protein).
3. PBS buffer (pH 7.2–7.4).

Important Notes

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. Optimal antibody concentration is 2 mg/mL. Concentrations below 1 mg/mL may reduce labeling efficiency; excessive concentration results in a very small antibody volume, which is unfavorable for purification recovery.
4. The sample volume for the centrifugal desalting column must be between 400 and 700 µL to ensure optimal performance. Volumes outside this range may lead to reduced antibody recovery or incomplete removal of unconjugated reagent.
5. Desalting columns are recommended for single use only.
6. Before using the kit, allow all components to equilibrate to room temperature.
7. All fluorescent dyes are prone to quenching. Please protect from light to minimize fluorescence quenching.

Instruction for use

1 Prepare the buffer

1.1 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

2.1 If the antibody concentration is ≥2 mg/mL in a suitable buffer, dilute it to 2 mg/mL and add 10% of the antibody volume of 1 M $NaHCO_3$ buffer. If the antibody is a lyophilized powder from an appropriate buffer, prepare a 2 mg/mL antibody solution by adding an appropriate volume of 0.1 M $NaHCO_3$ buffer. To prepare 0.1 M $NaHCO_3$ buffer, dilute the 1 M $NaHCO_3$ buffer 10-fold with ultrapure water.

2.2 Take one vial of reactive dye, add 10 µL DMSO, and vortex until the dye is dissolved.

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

2.3 Transfer 0.5 mL of the antibody solution into the reactive dye vial. Cap the vial and gently invert several times to mix the dye and antibody.

2.4 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 antibody.

3 Prepare the spin column

3.1 Place the Empty Spin Column on a column rack, open the cap, and remove the red bottom cap.

3.2 Resuspend the purification resin by inverting the bottle repeatedly. Then take 3.0 mL of the suspension and add it to the column. Allow the resin to settle naturally; the buffer in the column will drain by gravity. Initially, slight pressure may be needed to initiate flow. Centrifuge the column at 1,000 × g for 2 minutes, discard the storage buffer, and place the column back into the same collection tube.

Note: The purification resin is supplied as a 20% ethanol suspension, with a resin-to-ethanol volume ratio of 3:1 (v/v).

3.3 Add 2 mL of PBS to the top of the resin bed, centrifuge at 1,000 × g for 2 minutes, and discard the flow-through. Repeat this step a total of three times. The column is now equilibrated.

Note: When using a fixed-angle rotor, mark the side of the column opposite to the rotor center. For all subsequent centrifugation steps, place the column into the centrifuge with the marked side facing away from the rotor center.

4 Purify the labeled antibody

4.1 Carefully transfer the reaction solution from the vial onto the column. Cap the column lightly (do not overtighten).

4.2 Centrifuge the column at 1,000 × g for 2 minutes. The flow-through collected in the tube is the desired antibody conjugate.

5 Determine the Degree of Labeling (Optional)

The degree of labeling (DOL) is a useful parameter for characterizing the average number of label molecules that have covalently bonded to your sample protein during the labeling reaction. It can be determined from the absorption spectrum of your labeled bioconjugate.

5.1 Detect the absorbance of the antibody conjugate at 280 nm (A_{280}) and the absorbance maximum (λ_{max}) for the respective dye (A_{dye}). The λmax values for commonly used fluorophores are given in the table below.

Cat.No.	Dyes	λ_{max}(nm)	Em(nm)	ε_{dye}($M^{-1}cm^{-1}$)	CF_{280}	Target DOL	MW(g/mol)
A1491956	AF350	343	441	19,000	0.19	4-9	410
A1491957	AF405	401	422	34,000	0.7	4-9	1028
A1491958	AF488	490	525	76,000	0.1	4-9	1081
A1491959	AF532	530	555	81,000	0.09	4-9	723
A1491960	AF555	553	568	150,000	0.08	4-8	1250
A1491961	AF594	590	618	92,000	0.56	4-9	820
A1491962	AF647	650	668	239,000	0.03	4-8	1300
A1491963	AF660	663	691	132,000	0.1	3-8	1100
A1491964	AF680	681	704	184,000	0.05	3-7	1150
A1491965	AF700	696	719	192,000	0.07	3-7	1400
A1491966	AF750	752	776	240,000	0.04	3-8	1300
F1491968	FITC	498	517	73,000	0.35	3-8	389
P1491969	Pacific Blue	410	455	46,000	0.2	4-9	339
O1491970	Oregon Green 488	498	526	76,000	0.12	3-8	509
T1491971	TRITC	557	576	100,000	0.34	1-3	443
C1491973	Cy3	554	566	150,000	0.07	3-5	734
C1491974	Cy5	647	665	250,000	0.03	3-5	754

5.2 The following formula can be used to calculate the antibody concentration:

Antibody concentration (mg/mL) $= (A_{280} - CF_{280} \times A_{dye})/1.4$

5.3 The following formula can be used to calculate the degree of labeling:

$$DOL = \frac{A_{dye}/\varepsilon_{dye}}{(A_{280} - CF_{280} \times A_{dye})/210000}$$

Note:

a) 210,000 is the molar extinction coefficient (Ec) in $M^{-1}cm^{-1}$ of IgG at 280nm.

b) A_{dye} is the absorbance at maximum (λ_{max}) for the respective dye.

c) CF_{280} is the correction factor for the effect of the fluorophore on absorbance at 280nm.

Storage

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.