

APC Conjugation Kit

A1491946

Storage: Room Temperature. 2-8°C. Protect from light. Do not freeze. For detailed storage information, please refer to the kit contents.

Introduction

APC Conjugation Kit uses a simple and quick process for APC conjugation of antibodies. The kit provides all the reagents, purification columns needed to label up to 450 µg of antibody, as well as a detailed step-by-step protocol.

The APC Labeling Kit is a SH-reactive labeling kit. The kit provides a Conjugation Reagent that reacts with primary amines (-NH₂) to introduce a SH group onto the antibody. Subsequently, Modified APC and sulfhydryl group on the antibody form a covalent bond during conjugation.

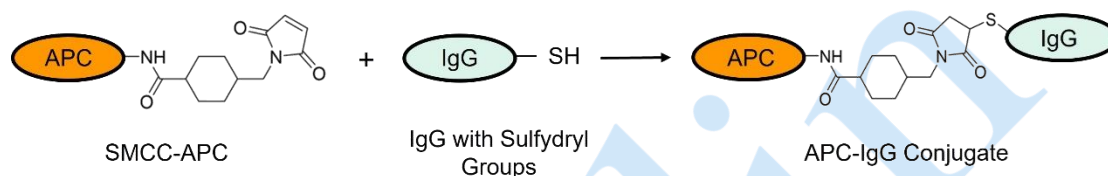


Fig. 1 APC Conjugation Kit (A1491946) Labeling Principle Schematic

Kit Contents

A1491946	Components	1 reaction	Storage	Quantity Per reaction
A1491946A	SMCC Activated APC	1 mg	2-8°C. Store in the dark. Do not freeze.	1 mg for labeling up to 450 µg of antibody.
A1491946B	Conjugation Buffer	10 mL	RT.	Prepare according to instructions.
A1491946C	Conjugation Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions.
A1491946D	Quencher Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions.
A1491946E	DMSO	1 mL	RT.	Prepare according to instructions.
A1491946F	Spin Desalting Column	1 EA	2-8°C. Do not freeze.	1 EA for 1 reaction.
A1491946G	Collection Tube	1 EA	RT.	1 EA for 1 reaction.

Instruction for use

1. Antibody sulfhydryl modification

To add sulfhydryls to antibodies: Use Conjugation Reagent, which modify primary amines. Ensure antibodies have a free sulfhydryl to react with the maleimide group of SMCC Activated APC.

1.1 Add Conjugation Buffer to dissolve the antibody to achieve a concentration of 3–5 mg/mL. A higher protein concentration is preferred. If less than 3 mg/mL, the antibody should be concentrated. If the IgG is not salt-free or if it is already in solution, the antibody must be transferred to the Conjugation Buffer via concentration exchange or dialysis.

1.2 Add 1 mL of purified water to the Conjugation Reagent and mix thoroughly. Note: The Conjugation Reagent solution must be prepared fresh before each use.

1.3 Add 4.13 μ L of prepared Conjugation Reagent per 450 μ g of antibody solution, briefly vortex to mix. Incubate at room temperature for 30 min.

1.4 During the incubation prepare a desalting column.

1.4.1 Snap open the bottom of the column and insert the column into matching collection tube. Remove and discard the column cap and centrifuge for 2 min at 1000 $\times$ g. Discard eluate. 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.

1.4.2 Apply 500 μ L of Conjugation Buffer and centrifuge for 2 min at 1000 $\times$ g. Discard eluate.

1.4.3 Repeat step 1.4.2 for two additional times. Add Conjugation Buffer for the third wash but wait to centrifuge until immediately prior to proceeding to step.

1.4.4 After the third wash check there is no solution in the column, if required briefly centrifuge until no solution remains.

1.5 Insert prepared desalting column into a fresh Collection Tube and apply the entire sample from step 1.3 to the column.

1.6 Centrifuge at 1000 $\times$ g for 2 min. Retain the eluate, which is the processed antibody.

2. Conjugate with SMCC Activated APC

2.1 Prepare APC solution (5 mg/mL): Add 200 μ L of purified water to SMCC Activated APC. Vortex gently until the solution is homogenous. Note: The Reconstituted SMCC Activated APC solution must be prepared fresh before each use.

2.2 Add the prepared antibody from step 1.6 to the prepared SMCC Activated APC solution. Incubate the mixture at room temperature for 1 hour and keep away from light.

2.3 Block excess free thiols

2.3.1 Add 500 μ L of DMSO into the Quencher Reagent. Note: The Quencher Reagent solution must be prepared fresh before each use.

2.3.2 Add 2.5 μ L of prepared Quencher Reagent into conjugation mixture from Step 2.2 and mix completely. Incubate at room temperature for 20 min and keep away from light.

2.4 Conjugation is complete. The reaction mixture should contain mostly the APC-antibody conjugate, with small amounts of free APC and antibody. Unconjugated APC should not interfere with the assay.

Storage

1. Store the APC-antibody conjugate at concentration > 0.5 mg/mL or add 1-10 mg/mL of BSA as a stabilizer.
2. Add preservative (e.g. 0.05% ProClin 300).
3. Store the conjugate solution at 4°C in the dark for two months. DO NOT FREEZE.
4. If there is aggregation in the conjugate solution, centrifuge briefly for 30 sec and use the supernatant only.

Matters needing attention

1. Any primary amines in the IgG solution (e.g., Tris or glycine) will interfere with conjugation. If necessary, dialyze or desalt samples into an appropriate buffer, before use.
2. Before formal experimentation, allow all reagents to reach room temperature, as lower temperatures may impair conjugation efficiency.
3. Do not reuse the purification resin.
4. The reactive dyes should be protected from prolonged exposure to light.
5. During operation, always wear a lab coat, disposable gloves, and protective equipment.
6. All products are for research use only.