

PerCP-Cy5.5 Conjugation Kit

P1491955

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

Introduction

PerCP-Cy5.5 Conjugation Kit uses a simple and quick process for PerCP-Cy5.5 conjugation of antibodies. The kit provides all the reagents, purification columns needed to label up to 100 µg of antibody, as well as a detailed step-by-step protocol.

The PerCP-Cy5.5 Conjugation 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 PerCP-Cy5.5 and sulfhydryl group on the antibody form a covalent bond during conjugation.

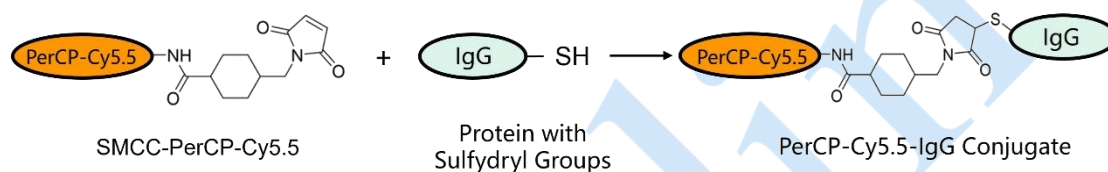


Fig. 1 PerCP-Cy5.5 Conjugation Kit (P1491955) Labeling Principle Schematic

Kit Contents

P1491955	Component	1 reaction	Storage	Quantity Per reaction
P1491955A	SMCC Activated PerCP-Cy5.5	0.1 mg	2-8°C. Store in the dark. Do not freeze.	0.1 mg for labeling up to 100 µg of antibody
P1491955B	Conjugation Buffer	10 mL	RT.	Prepare according to instructions
P1491955C	Conjugation Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions
P1491955D	Quencher Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions
P1491955E	DMSO	1 mL	RT.	Prepare according to instructions
P1491955F	Spin Desalting Column	1 EA	2-8°C. Do not freeze.	1 EA for 1 reaction
P1491955G	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 PerCP-Cy5.5.

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 1 μ L of prepared Conjugation Reagent per 100 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 Loosen the cap on a spin column and insert the column into matching collection tube. Snap open the bottom of the column 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. If the volume is less than 60 μ L, top up with Conjugation Buffer.

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

2. Conjugate with SMCC activated PerCP-Cy5.5

2.1 Add the prepared antibody from step 1.6 to the SMCC Activated PerCP-Cy5.5 powder and mix thoroughly. Incubate the mixture at room temperature for 1 hour and keep away from light.

2.2 Block excess free thiols

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

2.2.2 Add 1 μ L of prepared Quencher Reagent into conjugation mixture from Step 2.1 and mix completely. Incubate at room temperature for 20 min and keep away from light.

2.3 Conjugation is complete. The reaction mixture should contain mostly the PerCP-Cy5.5-antibody conjugate, with small amounts of free PerCP-Cy5.5 and antibody. Unconjugated PerCP-Cy5.5 should not interfere with the assay.

Storage

1. Store the PerCP-Cy5.5-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, the reagents must be returned to room temperature and centrifuged before use. Both lower temperatures and reagent residue on the centrifuge tube walls 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.