

PE-Cy5 Conjugation Kit

P1491952

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

Introduction

PE-Cy5 Conjugation Kit uses a simple and quick process for PE-Cy5 conjugation of antibodies. The kit provides all the reagents, purification columns needed to label up to 300 µg of antibody, as well as a detailed step-by-step protocol.

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

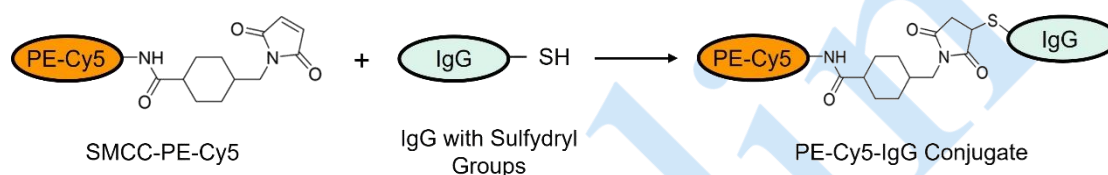


Fig. 1 PE-Cy5 Conjugation Kit (P1491952) Labeling Principle Schematic

Kit Contents

P1491952	Components	1 reaction	Storage	Quantity Per reaction
P1491952A	SMCC Activated PE-Cy5	1 mg	2-8°C. Store in the dark. Do not freeze.	1 mg for labeling up to 300 µg of antibody.
P1491952B	Conjugation Buffer	10 mL	RT.	Prepare according to instructions.
P1491952C	Conjugation Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions.
P1491952D	Quencher Reagent	2 mg	2-8°C. Do not freeze.	Prepare according to instructions.
P1491952E	DMSO	1 mL	RT.	Prepare according to instructions.
P1491952F	Spin Desalting Column	1 EA	2-8°C. Do not freeze.	1 EA for 1 reaction.
P1491952G	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 PE-Cy5.

- 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 2.8 μ L of prepared Conjugation Reagent per 300 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 PE-Cy5 Maleimide
 - 2.1 Prepare PE-Cy5 solution (5 mg/mL): Add 200 μ L of purified water to SMCC Activated PE-Cy5. Vortex gently until the solution is homogenous. Note: The Reconstituted SMCC Activated PE-Cy5 solution must be prepared fresh before each use.
 - 2.2 Add the prepared antibody from step 1.6 to the prepared PE-Cy5 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 PE-Cy5-antibody conjugate, with small amounts of free PE-Cy5 and antibody. Unconjugated PE-Cy5 should not interfere with the assay.

Storage

1. Store the PE-Cy5-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.