

ZymeBright™ PE Site-Specific Antibody Conjugation Kit

Details

ZymeBright™ PE Site-Specific Antibody Conjugation Kit is an enzymatic method for the conjugation of PE to the Fc region of Rabbit IgG, Rat IgG1 & IgG2a, Human IgG1-4, or Mouse IgG1 & IgG3 for the use in fluorescent immunoassays. The expected degree of labeling (F/P ratio) of antibodies conjugated with this kit is 2.

For buffer compatibility, please refer to the product website of PK30080.

Kit Contents (Cat No. PK30080)		
Component	Cat No.	Amount
Antibody Modifier Reagent (Vial A)	PK3007X-A	5 µL
Lyophilized Modified PE (Vial B)	PK30080-B	1 rxn
Lyophilized PTGase (Vial C)	PK3007X-C	3 mg (provided in excess)
Conjugation Buffer (Vial D)	PK3007X-D	200 µL
Lyophilized Quenching Reagent (Vial E)	PK3007X-E	5 mg
0.6 mL Low Adhesion Tube	PK3007X-F	2 tubes

General Guidelines:

Storage: Store at -20°C for up to 1 year. After opening, store at 2-8°C. Protect from light and moisture. Reagents must be used within 1 week of opening.

Required Materials:

Orbital Thermal Mixer

Pipettes

Sterile PBS

Prior to Beginning:

Antibody should be at 1 mg/mL in compatible buffer. Please refer to the product website for buffer component compatibility.

The entire conjugation protocol should be performed in sterile conditions to avoid contamination. Lyophilized modified PE should be protected from light throughout.

Spin down Vials A, C, D, and E. 10-15 minutes prior to beginning, bring the Lyophilized Modified PE (Vial B) and Conjugation Buffer (Vial D) to room temperature. Store Quenching Reagent (Vial E) at 2-8°C. Preheat the orbital thermal mixer to 37°C.

On day 2, bring Quenching Reagent (Vial E) to room temperature 10-15 minutes prior to beginning.

Protocol

Day 1: Antibody Modification

1. Add 100 µL antibody (at 1 mg/mL) to a provided 0.6 mL low adhesion tube.
2. Add 2 µL Antibody Modifier Reagent (Vial A) to antibody in 0.6 mL low adhesion tube. Pipette for 10-15 seconds with a 200 µL or 1000 µL pipette to fully mix.
3. Incubate in a 3 mm orbital thermal mixer at 37°C, 800 rpm for 30 minutes. If using a 2 mm orbital thermal mixer, mix at 1000 rpm.
Note: Avoid prolonged incubation of the antibody with the Modifier Reagent.

Prepare Lyophilized Modified PE and PTGase

4. Transfer modified antibody solution from the 0.6 mL low adhesion tube into Vial B: Lyophilized PE. Pipette gently for 10-15 seconds to fully dissolve Lyophilized PE.
5. Pipette entire contents of Vial B into the other 0.6 mL low adhesion tube.
6. Add 600 µL sterile PBS to Vial C: Lyophilized PTGase and pipette for 10-15 seconds to fully dissolve Lyophilized PTGase.
Note: PTGase should be used within 1 hour of dissolving to avoid a decrease in enzymatic activity and reduction of conjugation efficiency.

Antibody Conjugation

7. Add 20 µL PTGase solution (Vial C) and 45 µL Conjugation Buffer (Vial D) to the 0.6 mL low adhesion tube from step 5. Pipette for 10-15 seconds to mix well.
8. Incubate in a 3 mm orbital thermal mixer at 37°C, 800 rpm for 18-24 hours. If using a 2 mm orbit thermal mixer, mix at 1000 rpm. Protect from light.

Day 2: Quench Conjugation Reaction

9. Add 100 µL sterile PBS to Vial E: Lyophilized Quenching Reagent. Pipette for 10-15 seconds to fully dissolve.
10. Add 83 µL Quencher Reagent Solution from Vial E to the conjugated antibody in the 0.6 mL low adhesion tube. Pipette for 10-15 seconds to mix.
11. Incubate at room temperature in the dark for 30 minutes.

Conjugated Antibody Storage

12. The final conjugated antibody concentration is approximately 0.4 mg/mL in 250 µL.
13. If desired, dilute conjugated antibody using sterile PBS and a preservative (for example sodium azide). Conjugated antibody with preservative is stable for at least 1 year stored at 4°C protected from light.
Note: A small amount of precipitation in the final conjugated antibody solution will occasionally occur and will not affect antibody performance.