HRP-Labeling Kit

Product Code: OM700049

Package Contents:

Reaction Components	Specification
A: Horseradish Peroxidase (HRP) Lyophilized Powder	1 vial
B: Labeling Buffer	1 bottle
C: Labeling Stop Reagent (Lyophilized Powder)	1 vial
D: Storage Solution	1 bottle

Storage Conditions: HRP lyophilized powder should be stored at -20°C. Other labeling components should be stored at 4°C. The labeling kit is transported with ice packs.

Product Description: This HRP Labeling Kit provides all the reagents required for rapid HRP conjugation. Utilizing an improved sodium periodate method, the pre-oxidized HRP can bind to free amino groups of the target molecule via Schiff's base reaction. It is designed for labeling antibodies, proteins, peptides, or other macromolecules containing primary amino groups (-NH_2). The HRP supplied in this kit is sufficient to label approximately 2 mg of IgG antibody. Users can calculate the required amount of HRP based on their specific needs.

Experimental Preparation:

1. Carefully read the entire user manual before starting.

2. If the concentration of the target molecule is too low, concentrate it to 2-4 mg/mL using ultrafiltration centrifugation. If the target molecule contains interfering substances such as free Tris, amino acids, or other amine-containing compounds, perform repeated ultrafiltration with PBS to ensure complete removal.

3. Remove all kit components from the refrigerator in advance and allow them to equilibrate to room temperature.

4. Completely dissolve Component A in 250 µL of deionized water. Calculate the required amount of HRP to be added based on the quantity of your target molecule (An IgG

antibody-to-HRP mass ratio between 2:1 and 1:1 is optimal; the ratio for other compounds should be determined on a case-by-case basis).

5. Completely dissolve Component C in 1 mL of deionized water to prepare the labeling stop solution.

Procedure: (This protocol is based on labeling 1 mg of antibody.)

1. Add 1/10 volume of Labeling Buffer (Component B) to the solution containing the target molecule. Mix gently by pipetting, avoiding bubble formation.

2. Add 125 µL of the reconstituted Component A solution to the mixture from Step 1. Mix gently by pipetting, avoiding bubble formation, and incubate for 2 hours at room temperature, protected from light.

3. Add 1/10 volume of the prepared Labeling Stop Solution to the reaction mixture from Step 2. Mix thoroughly and incubate for 1 hour at room temperature. **Note:** The labeling reaction is complete at this stage. The labeled protein can be preliminarily tested in ELISA and Western blot assays, although reaction by-products and hydrolyzed HRP remain in the solution.

4. After the stop reaction is complete, add an equal volume of Preservative Solution to the mixture. Mix thoroughly and store at -20°C for future use.

Notes:

1. This kit is suitable for labeling all macromolecules containing primary amino groups (-NH_2), such as proteins, antibodies, and peptides. The optimal amount of the target molecule should be determined based on its molecular weight and the desired degree of labeling.

2. The kit includes Component D (Preservative Solution), whose main component is glycerol. Users may also select an alternative preservation solution better suited to the characteristics and intended application of their specific labeled product.

3. The shelf life of the unopened kit is 3 months. Please use the product within this period.

4. The reconstituted Horseradish Peroxidase working solution (Component A) is best used immediately after preparation. Any remaining solution can be stored at -20°C for up to one week, but a reduction in conjugation efficiency may occur.