HRP Conjugation Kit (Periodate Activated)

P1508744

Storage: Room Temperature. -20℃. Protect from light. For detailed storage information, please refer to the kit contents.

SKU	Prod. Name	Size
P1508744	HRP Conjugation Kit (Periodate Activated)	0.1 mg
P1506712	HRP Conjugation Kit (Periodate Activated)	1 mg

Introduction

HRP Conjugation Kit (Periodate Activated) uses a simple and quick process for HRP conjugation of antibodies. The kit provides all the reagents needed to label to 100 μg of antibody, as well as a detailed step-by-step protocol.

This kit provides activated HRP with amine reactivity. The horseradish peroxidase (HRP) is activated via the sodium periodate oxidation method, which targets the glycosyl groups on HRP. The resulting activated HRP carries aldehyde groups that can spontaneously and efficiently cross-link with primary amines on antibodies or other proteins through a Schiff's base reaction. The kit is suitable for labeling substances containing free amino groups, such as antibodies, proteins, or small-molecule compounds bearing primary amines. It enables simple and rapid conjugation of antibodies with HRP. The kit offers excellent stability, is ready to use on demand, and ensures a convenient and efficient workflow.

Kit Contents

P1508744	Components	1 Reaction	Storage	Quantity Per reaction
P1508744A	Activated HRP	0.1 mg	-20℃. Store in the dark.	0.1 mg for labeling 100 μg of antibody.
P1508744B	Sodium Carbonate-Bicarbonate	1 vial	RT.	Prepare according to instructions.
P1508744C	Sodium Cyanoborohydride	1 vial	RT.	Prepare according to instructions.
P1508744D	Collection Tube	1 EA	RT.	1 EA for 1 reaction.

Instruction for use

1. Antibody Preparation

The recommended antibody concentration for labeling is 2–5 mg/mL. Labeling efficiency may be reduced if the antibody concentration is below 2 mg/mL. The antibody or protein to be labeled should be dissolved in an amine-free buffer (e.g., MES, MOPS, HEPES, or PBS) at a

pH of 6.5–8.5. The solution must be free of preservatives such as sodium azide, which can inhibit horseradish peroxidase (HRP) activity and interfere with downstream applications. If the antibody solution contains such interfering substances, it is recommended to exchange the buffer to 0.01 M PBS (pH 7.0–7.4) using dialysis or ultrafiltration prior to labeling.

2. Prepare the reagents

Prepare a 1 M Sodium Carbonate-Bicarbonate buffer (hereafter referred to as 1 M CB): Add 1 mL of ultrapure water to the Sodium Carbonate–Bicarbonate powder. Cap the tube and shake until the powder is completely dissolved to obtain 1 M CB. Prepare 0.1 M CB by diluting the 1 M solution 10-fold with ultrapure water. Note: The prepared Buffer is stable for 1-2 weeks when stored at 2-8°C.

3. Antibody-HRP Conjugation Procedure

3.1 Prepare HRP solution (5 mg/mL)—Add 20 µL of Ultrapure water to Activated HRP. Vortex gently until the solution is homogenous. Note: The Reconstituted SMCC Activated HRP solution must be prepared fresh before each use.

3.2 Add 100 µg of the prepared antibody to the prepared HRP solution. Add 1 M CB equivalent to 10% of the reaction volume (e.g., For a 100 µL reaction, add 10 µL of 1 M CB). Mix thoroughly and incubate at room temperature, protected from light, for 2 hours.

3.3 Preparation of Sodium Cyanoborohydride solution, Add 400 µL of 0.1 M CB to the Sodium Cyanoborohydride tube. Cap the tube tightly and gently vortex or invert until the powder is completely dissolved. Note: The Sodium Cyanoborohydride solution must be prepared fresh before each use.

3.4 Add sodium cyanoborohydride solution to the 3.2 mixture to a final concentration of 10% of the total volume. Incubate the reaction at 4°C in the dark for 30 minutes to obtain the conjugated antibody. For storage, the conjugate can be transferred to the Collection Tube.

Storage

Add 50% glycerol to the HRP-antibody conjugate and store at -20°C protected from light.

Matters needing attention

1. Upon receipt of this product, it should be used immediately or stored according to the recommended conditions. With prolonged storage, the enzymatic activity and labeling capability of the Activated HRP may decrease to some extent.
2. The optimal antibody quantity should be determined experimentally based on specific requirements. Generally, a higher HRP-to-antibody ratio increases labeling efficiency and the number of HRP molecules conjugated per antibody, leading to improved sensitivity and signal-to-noise ratio. However, excessive labeling may block the active sites of the antibody and reduce its immunoreactivity. Therefore, the HRP-to-antibody mass ratio can be optimized within the range of 0.5:1 to 2:1. We recommend starting with a mass ratio of 1:1 for most applications.
3. Before formal experimentation, allow all reagents to reach room temperature, as lower

temperatures may impair conjugation efficiency. Before opening the bottle cap, centrifuge it briefly to settle the powder at the bottom of the tube, preventing loss during opening.

4. During operation, always wear a lab coat, disposable gloves, and protective equipment.
5. All products are for research use only.