HRP Conjugation Kit (SMCC Activated)

M1508746

Storage: Room Temperature. 2-8℃. -20℃. Protect from light. For detailed storage information, please refer to the kit contents.

SKU	Prod. Name	Size
M1508746	HRP Conjugation Kit (SMCC Activated)	0.1 mg
H1506714	HRP Conjugation Kit (SMCC Activated)	1 mg

Introduction

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

The HRP Conjugation Kit (SMCC Activated) is a SH-reactive labeling kit. The kit provides a Conjugation Reagent that reacts with primary amines (-NH_2) to introduce a SH group onto the antibody. Subsequently, Modified HRP and sulfhydryl group on the antibody form a covalent bond during conjugation.

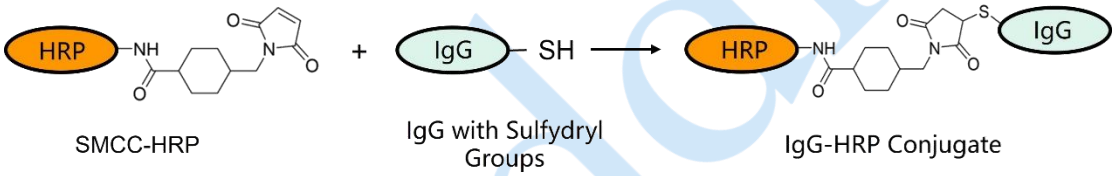

Fig. 1 HRP Conjugation Kit (SMCC Activated) (M1508746) Labeling Principle Schematic

Kit Contents

M1508746	Components	1 reaction	Storage	Quantity Per reaction
M1508746A	SMCC Activated HRP	0.1 mg	-20℃. Store in the dark.	0.1 mg for labeling 100 μg of antibody.
M1508746B	Conjugation Buffer	10 mL	RT.	Prepare according to instructions.
M1508746C	Conjugation Reagent	2 mg	2-8℃. Do not freeze.	Prepare according to instructions.
M1508746D	Quencher Reagent	2 mg	2-8℃. Do not freeze.	Prepare according to instructions.
M1508746E	DMSO	1 mL	RT.	Prepare according to instructions.
M1508746F	Spin Desalting Column	1 EA	2-8℃. Do not freeze.	1 EA for 1 reaction.
M1508746G	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 HRP.

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 µL of prepared Conjugation Reagent per 100 µg of antibody solution, briefly vortex to mix.

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×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 µL of Conjugation Buffer and centrifuge for 2 min at 1000×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 µL, top up with Conjugation Buffer.

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

2. Conjugate with SMCC activated HRP

2.1 Add the prepared antibody from step 1.6 to the SMCC Activated HRP 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 µL of DMSO into the Quencher Reagent. Note: The Quencher Reagent solution must be prepared fresh before each use.

2.2.2 Add 1 µL 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 HRP-antibody conjugate, with small amounts of free HRP and antibody. Unconjugated HRP should not interfere with the assay.

Storage

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

Matters needing attention

1. The antibody or protein to be labeled should be dissolved in an amine-free buffer (e.g., MES, MOPS, HEPES, PBS) at a pH of 6.5–8.5. The solution must be free of preservatives such as sodium azide. If present, these must be removed via purification prior to the labeling reaction.
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. 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.
5. During operation, always wear a lab coat, disposable gloves, and protective equipment.
6. All products are for research use only.