HRP Conjugation Kit (SMCC Activated)

H1506714

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 1 mg 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.

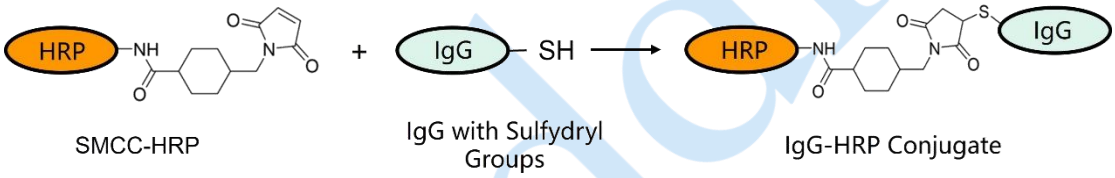

Fig. 1 HRP Conjugation Kit (SMCC Activated) (H1506714) Labeling Principle Schematic

Kit Contents

H1506714	Components	1 reaction	Storage	Quantity Per reaction
H1506714A	SMCC Activated HRP	1 mg	-20℃. Store in the dark.	1 mg for labeling 1 mg of antibody.
H1506714B	Conjugation Buffer	20 mL	RT.	Prepare according to instructions.
H1506714C	Conjugation Reagent	2 mg	2-8℃. Do not freeze.	Prepare according to instructions.
H1506714D	Quencher Reagent	2 mg	2-8℃. Do not freeze.	Prepare according to instructions.
H1506714E	DMSO	1 mL	RT.	Prepare according to instructions.
H1506714F	Empty Spin Column	1 EA	RT.	1 EA for 1 reaction.
H1506714G	Desalting Resin	2 mL	2-8℃. Do not freeze.	2 mL for 1 reaction.
H1506714H	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 9.17 µL of prepared Conjugation Reagent per 1 mg of antibody solution, briefly vortex to mix.

1.4 During the incubation prepare a desalting column.

1.4.1 Suspend the Desalting Resin by repeated inversion of the apped bottle.

1.4.2 Assembly of the desalting column: Open the Empty Spin Column and transfer the Desalting Resin slurry into the column. Allow the resin to settle as the buffer drains. Then add 2 mL of Conjugation Buffer to the original resin bottle to resuspend any residual resin, and transfer the resulting suspension into the Empty Spin Column. The desalting column is now fully assembled. Note: The Desalting Resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

1.4.3 Place the desalting column into the matching collection tube. Open 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.

1.4.4 Apply 2 mL of Conjugation Buffer and centrifuge for 2 min at 1000×g. Discard eluate.

1.4.5 Repeat step 1.4.4 for two additional times. Add Conjugation Buffer for the third wash but wait to centrifuge until immediately prior to proceeding to step.

1.4.6 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. Note: Before adding the sample, ensure that no residual eluate remains in the collection tube.

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 Prepare HRP solution (10 mg/mL)—Add 100 µL of purified water to SMCC Activated HRP. Vortex gently until the solution is homogenous. Note: The Reconstituted SMCC Activated HRP solution must be prepared fresh before each use.

2.2 Transfer the prepared antibody from step 1.6 and the prepared HRP solution to the collection tube and mix thoroughly. 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 µL of DMSO into the Quencher Reagent. Note: The Quencher Reagent solution must be prepared fresh before each use.

2.3.2 Add 4 µL 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 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.