

BSA Conjugation Kit (EDC, 2mg)

B1509080

Storage: 2-8°C. Store in the dark. For detailed storage information, please refer to the kit contents.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

Bovine serum albumin (BSA) is a serum albumin protein that has numerous biochemical applications including ELISA (Enzyme-Linked Immunosorbent Assay), immunoblots, and immunohistochemistry. Like most abundant plasma proteins, it is very stable and soluble. In addition, the 67 kDa protein is sufficiently large and complex to be fully immunogenic. It contains numerous sites per molecule for effective conjugation of peptides and other antigens using amine-reactive or carboxyl-reactive crosslinkers. Consequently, BSA is a popular carrier protein for conjugation to haptens and other weak antigens to make them more immunogenic for antibody production.

This kit is designed for the coupling of haptens to carrier proteins, utilizing the primary amine groups of the hapten. This kit utilizes the chemical heterobifunctional crosslinker EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride). The resulting conjugates can stimulate immune responses in organisms for the production of hapten-targeted antibodies. The carboxyl-reactive carbodiimide reacts with exposed carboxyl and amino groups on haptens to form stable bonds. This kit contains BSA and dedicated buffers compatible with carbodiimide reactions, as well as desalt spin columns that enable highly efficient protein recovery via a simple centrifugation step. This kit is available in two sizes, supporting either 1 or 5 independent conjugation reactions.

Required materials not supplied

1. Microcentrifuge capable of 1000 × g.
2. Desired hapten for labeling.
3. PBS buffer (pH 7.2-7.4).

Contents and storage

B1509080	Component	Appearance	1 reaction	5 reactions	Storage	Quantity Per reaction
B1509080A	Bovine Serum Albumin	Lyophilized powder	1 vial	5 vial	2-8°C. Store in the dark Do not freeze	1 vial for conjugating 2 mg of hapten
B1509080B	EDC	White powder	1 vial	5 vial	2-8°C	1 vial for 1 reaction
B1509080C	EDC Conjugation Buffer	Colorless clear liquid	4 mL	20 mL	2-8°C	Prepare according to instructions
B1509080D	Purification Buffer	Colorless clear liquid	10 mL	50 mL	2-8°C	Prepare according to instructions
B1509080E	Purification Resin	White bead slurry	3 mL	15 mL	2-8°C, Do not freeze	3 mL for 1 reaction
B1509080F	Empty Spin Column	Transparent tube	1 EA	5 EA	2-8°C	1 EA for 1 reaction

Important

1. The following protocol is a general protocol for a wide variety of haptens. Optimize the protocol accordingly for the conjugation efficiencies based on the size and structure of your hapten.
2. Using a molar excess of hapten over carrier protein ensures efficient conjugation. In general, a reaction with equal mass amounts of hapten and carrier protein will achieve sufficient molar excess.
3. In some cases precipitation may occur. The precipitation occurs due to excessive hapten-carrier protein conjugation. The precipitation may be minimized by reducing the amount of EDC in the reaction.
4. If DMSO was used in the conjugation, prepare the Purification Buffer Salts with the same percentage of DMSO used for the conjugation. This minimizes precipitation in the column during desalting.
5. If a precipitate formed during conjugation, centrifuge the precipitated material, collect the supernatant and save the precipitate. Purify only the supernatant. Combine the purified conjugate to the precipitate.
6. Do not reuse the purification resin.
7. Before formal experimentation, the reagents must be returned to room temperature and centrifuged before use. Both lower temperatures and reagent residue on the centrifuge

tube walls may impair conjugation efficiency.

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

Instruction for use

1. Prepare BSA solution (10 mg/mL): Add 200 μ L of EDC Conjugation Buffer into the vial of Bovine Serum Albumin (BSA) to make a 10 mg/mL solution.
2. Prepare hapten solution (5 mg/mL): Dissolve 2 mg hapten in 400 μ L EDC Conjugation Buffer.

Note: For haptens with limited solubility, use $\leq 30\%$ DMSO in the final conjugation solution. Using a higher concentration of DMSO might irreversibly denature the carrier protein.

3. Add the 400 μ L of hapten solution to the 200 μ L BSA solution to have Hapten-BSA mixture solution.
4. Add the Hapten-BSA solution from step 3 into one vial of EDC, dissolve it by gentle mixing. Incubate at room temperature for 2 hours.

Note: During the incubation period, proceed to steps 5 below, to prepare a spin column for the purification of the labeled protein.

5. Prepare the spin column

- 5.1 Place an empty spin column in a 15 mL collection tube.
- 5.2 Suspend the purification resin by repeated inversion of the reagent bottle. Then add 3.0 mL of the suspension into the column and allow the resin to settle. Allow the column storage buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Centrifuge the column at $1000 \times g$ for 2 minutes, discard the storage buffer and return column to the same collection tube.

Note: The purification resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

- 5.3 Add 2 mL of PBS Buffer, then centrifuge the column-tube assembly at $1000 \times g$ for 2 minutes to equilibrate the column.

Note: If the conjugate is to be used for injection within one week, PBS may be used for purification. If the conjugate will be frozen, use the Purification Buffer for purification, which will preserve the product during freeze-thaw cycles.

- 5.4 Repeat step 5.3 for two additional times. Add PBS for the third wash but wait to centrifuge until immediately prior to proceeding to step.

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.

- 5.5 After the third wash check there is no solution in the column, if required briefly centrifuge until no solution remains.

6. Purification with Desalting Column

- 6.1 Place column into a new 15 mL collection tube and apply the entire sample from step 4 to

the column.

Note: If a precipitate formed during conjugation, centrifuge the precipitated material, collect the supernatant and save the precipitate. Purify only the supernatant. Combine the purified conjugate to the precipitate.

6.2 Centrifuge the column-tube assembly at $1000 \times g$ for 2 minutes. The purified conjugate is in the collection tube.

7. Storage

7.1 The hapten-carrier conjugate can now be used for immunization. If the hapten-carrier conjugate is to be stored for more than a few days, sterile filter the conjugate and store in a sterile container at 4°C or -20°C .

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