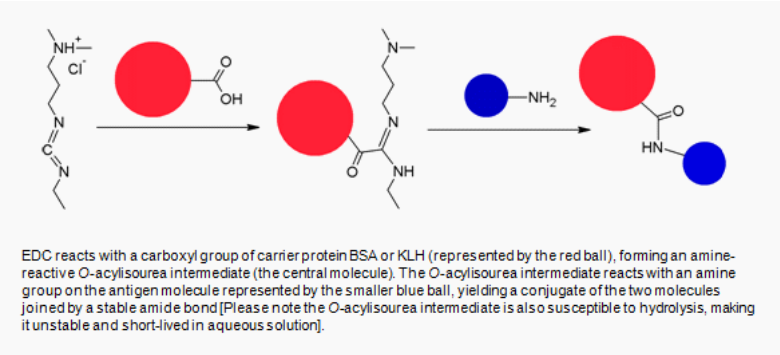
ReadiLink™ BSA Conjugation kit *For Antibody Development*

Ordering Information:	*Storage Conditions:*
Product Number: 5501	Store at 4 °C

Introduction

Bovine serum albumin (BSA) is a serum albumin protein that has numerous biochemical applications including ELISAs (Enzyme-Linked Immunosorbent Assay), immunoblots, and immunohistochemistry. Like most abundant plasma proteins, it is very stable and soluble in aqueous media. 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 ReadiLink™ BSA Conjugation kit is primarily optimized for the simple preparation of hapten-carrier conjugates for immunization and antibody production.

The ReadiLink™ BSA Conjugation kit is one-step conjugation of a hapten to a carrier protein using the carboxyl-reactive carbodiimide as the crosslinker. The resulting conjugate is used for eliciting an immune response and antibody production against the hapten. The carboxyl-reactive carbodiimide reacts with exposed carboxyl and amino groups on peptides and proteins to form stable bonds. These kits contain BSA formulated in buffers compatible with the carboxyl-reactive carbodiimide reactions and desalt spin columns, which offer exceptional protein recovery by simple centrifugation step.



EDC reacts with a carboxyl group of carrier protein BSA or KLH (represented by the red ball), forming an amine-reactive *O*-acylisourea intermediate (the central molecule). The *O*-acylisourea intermediate reacts with an amine group on the antigen molecule represented by the smaller blue ball, yielding a conjugate of the two molecules joined by a stable amide bond [Please note the *O*-acylisourea intermediate is also susceptible to hydrolysis, making it unstable and short-lived in aqueous solution].

Kit Components

Components	Amount	Handling and Storage
A. BSA (bovine serum albumin)	2 X 2mg	Store at 4 °C
B. Conjugation Buffer (pH 4.7)	20 mL	Store at 4 °C
C. EDC (1-ethy 1-3-[dimethylaminopropyl] carbodiimide hydrochloride)	2 X 10 mg	Store at 4 °C
D. Purification Buffer Salts (pH 7.2)	2 Bottles	Store at 4 °C
E. Spin Desalting Columns (7K MWCO)	2 X 2 mL	Store at 4 °C. Do not freeze

Conjugation Protocol

Brief Summary

Prepare protein solution → Prepare hapten solution→ Mix protein with hapten into EDC → Incubate the reaction at RT for 2 hr→ Purify the conjugate by desalting

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The following protocol is a general protocol for a wide variety of haptens. Optimize the protocol accordingly for the conjugation efficiencies upon the size and structure of your hapten. 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.

1. Prepare BSA-Hepten Conjugation:

1.1 Add 200 µL of ddH_2O into the vial of BSA (Component A) to make a 10 mg/mL solution.
1.2 Dissolve up to 2 mg hapten in 450 µL Conjugation Buffer (Component B).
Note: Some haptens might have limited solubility, use DMSO (≤ 30% in the final conjugation solution) to dissolve it first. Higher concentration of DMSO might irreversibly denature the carrier protein.
1.3 Add the 450 µL hapten solution (from Step 1.2) into the 200 µL BSA solution (from Step 1.1) to have Hapten BSA mixture solution.
1.4 Add the Hapten-BSA solution (from Step 1.3) into one vial of EDC (10mg), dissolve it by gentle mixing. Incubate at room temperature for 2 hours.
1.5 Purify the conjugate by desalting to remove non-reacted crosslinker and protein preservative (e.g., sodium azide).

2. Purify BSA-Hepten conjugate:

2.1 Add 10 mL of degassed ultrapure H_2O into one bottle of Purification Buffer Salts (Component D), mixing well. Unused buffer can be stored at 4 °C for 1 week.
2.2 Twist off the bottom closure of the desalting column (Component E), and loosen the cap. Place the column in a collection tube.
2.3 Centrifuge the column at 1,000g for 2 minutes to remove the storage solution.
2.4 Remove the cap and slowly add 1 mL of purification buffer to the column. Centrifuge at 1,000g for 2 minutes, remove the buffer. Repeat this step for 3 additional times, discarding the buffer from the collection tube.
2.5 Place the column to a new collection tube, and gently apply the sample into the center of the compact resin bed.
2.6 Centrifuge the column at 1,000g for 2 minutes to collect the sample.
2.7 The Hapten-BSA conjugate can now be used for immunization. If the Hapten-BSA conjugate is to be stored for more than a few days, sterile filter the conjugate, and store at 4 °C or - 20 °C.
Note 1: If the conjugate is to be used within one week, PBS may be used for purification. If the conjugate will be frozen, use the purification buffer salts (Component D) for purification.
Note 2: If DMSO is used in the conjugation, prepare the purification buffer salts with the same percentage of DMSO used for conjugation. This will minimize the precipitation in the column during desalting.
Note 3: If a precipitate formed during conjugation, centrifuge the precipitated material, collect the supernatant and save the precipitate. Purify the supernatant. Combine the precipitate and the purified conjugate.

References

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2. Enomoto H, Li CP, Morizane K, Ibrahim HR, Sugimoto Y, Ohki S, Ohtomo H, Aoki T. (2008) Improvement of functional properties of bovine serum albumin through phosphorylation by dry-heating in the presence of pyrophosphate. J Food Sci, 73, C84.
3. Ledesma-Osuna AI, Ramos-Clamont G, Vazquez-Moreno L. (2008) Characterization of bovine serum albumin glycated with glucose, galactose and lactose. Acta Biochim Pol, 55, 491.
4. Wang JH, Wang HQ, Zhang HL, Li XQ, Hua XF, Cao YC, Huang ZL, Zhao YD. (2007) Purification of denatured bovine serum albumin coated CdTe quantum dots for sensitive detection of silver(I) ions. Anal Bioanal Chem, 388, 969.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest®. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.