

# BSA-SUCC

BSA Protein SUCC

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BSA-SUCC proteins are prepared by increasing the number of reactive carboxylic functions on the surface of the carrier protein. Amino groups are modified with Succinic anhydride inducing a rather « negative » molecule, since the positive charged amino groups are masked by negative charged from carboxylic group resulting in a significant PI change

## ENHANCED CARBOXYLIC DISPLAYING CARRIER PROTEINS

BSA-SUCC proteins display increased reactive Carboxylic functions on their surface useful for covalent binding to the appropriate functional groups:

amino group function  
Sulfhydryl function  
Hydrazides function

The introduction of carboxylates affects the overall charge characteristics or pI of the molecule being derivatized. The modification of amines residues by acylation with anhydrides not only eliminates the positive charge contribution of the protonated amine, but also adds the negative charge contribution of the acid. The results may be a change of minus two in net charge per group modified. While the reaction involved in such derivatizations are conducted under relatively mild conditions, severe alterations in net charge may cause some macromolecules, like proteins, to denature or lose activity.

## HAPTEN COUPLING TO BSA-SUCC

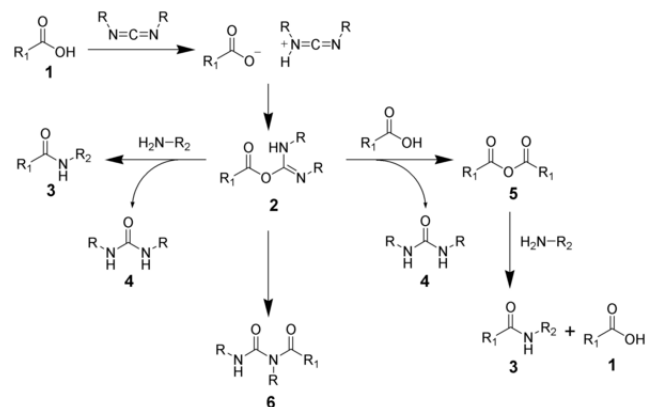
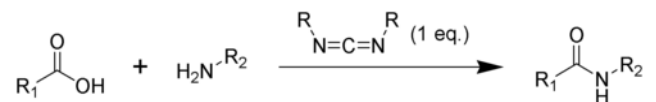
BSA-SUCC protein activated use carbodiimide crosslinker react with haptens contain amino group and preserve the native chemical structure of hapten.

**BSA-SUCC** increases the amount of reactive carboxylic functions on protein surface by 100% and enhanced linkage between amino group from hapten with carboxylic group from protein carrier.

N-substituted carbodiimides can react with carboxylic acids in protein surface to form highly reactive, O-acylisourea derivatives that are extremely short-lived. This active species then can react with a nucleophile such as a primary amine to form an amide bond. Other nucleophiles are also reactive. Sulfhydryl groups may attack the active species and form thioester linkage, although these are not as stable as the bond formed with an amine.

Hydrazide containing compounds also can be coupled to carboxylate groups using a carbodiimide mediated reaction. Using bifunctional hydrazide reagents, carboxylates can be modified to possess terminal hydrazide groups able to conjugate with other carbonyl compounds.

In addition, oxygen atoms may act as the attacking nucleophile, such as those in water molecules. In aqueous solutions, hydrolysis by water is the major competing reaction, cleaving off the activated ester intermediate, forming an isourea, and regenerating the carboxylate group.



reaction.

EDAC water soluble is perhaps the most carbodiimide for use in conjugating haptens containing amino group with proteins containing carboxylic function.

Excess reagent and the isourea formed as the product of reaction are both water soluble and may be easily removed by dialysis or gel filtration.

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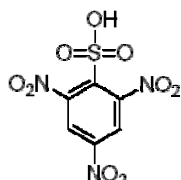
LOT# BSASUC2307

CATALOGUE# R6003

10 mg DRY FREEZE MATERIAL

## BSA-SUCC QUALITY CONTROL

Quality control is based in comparing the response of BSA-SUCC and native BSA when TNBS (Tri-nitro benzene sulfonate) test was applied, aimed at determining the number of reactive amine moieties. In the TNBS test the response is proportional to the amount of reactive amine functions. **BSA-SUCC not show reaction with TNBS reagent compare with native BSA.**



Molecules containing primary amine groups can react with 2,4,6-trinitrobenzenesulfonate (TNBS) to form a highly chromogenic derivative.

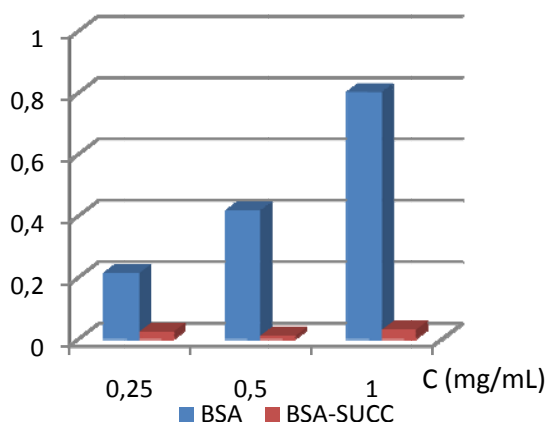
This reaction may be used to assay the amine content of compounds by measuring the absorbance of the orange-colored product.

TNBS has been used to measure the free amino groups in proteins (Sashidhar, R. B.; Capoor, A. K.; and Ramana, D.; (1994) Quantitation of amino group using aminoacids as reference standards by trinitrobenzene sulfonic acid. J. Immunol. Methods 167, 121-127)

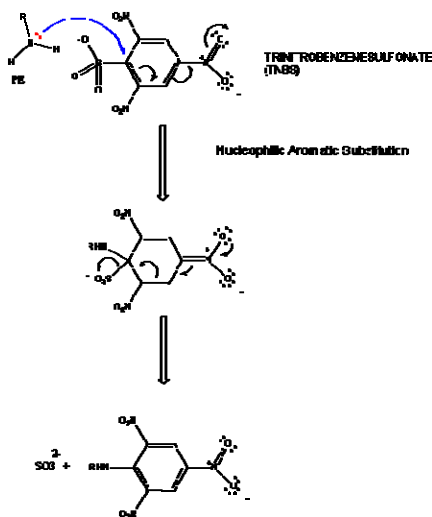
C (mg/mL)	0.25	0.50	1.00
BSA	0.214	0.416	0.802
BSA SUCC	0.024	0.010	0.030

(BSA-SUCC and BSA Absorbance (420nm) obtained using TNBS reaction)

Carbodiimide – mediate amide bond formation effectively occurs between pH 4,5 and 7,5. Buffer systems using MES may be used to stabilize the pH during the course of



- [1] Yamada, H.; Imoto, T.; Fujita, K.; Okazaki, K.; Motomura, M.; (1981) Selective modification of aspartic acid-101 in lysozyme by carbodiimide reaction, Biochemistry 20, 4836 – 4842..
- [2] F.S. Chu, H.P. Lau, T.S. Fan and G.S. Zhang. Ethylene diamine modified Bovine Serum Albumin as Protein carrier in the production of antibody against mycotoxins (1982). J. Immunol. Methods, 55, p. 73-78
- [3] Williams, A.; Ibrahim, I. A.; 1981, A mechanism involving cyclic tautomers for the reaction with nucleophiles of the water soluble peptide coupling reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC); J. Am. Chem. Soc.; 103, 7090 - 7095.



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