HOOK™ Maleimide Activated Streptavidin

For conjugation of Streptavidin to sulfhydryl groups containing proteins, peptides and ligands

(Cat. #786-1653, 786-1654)

INTRODUCTION

Streptavidin is a non-glycosylated 52.8 kD tetrameric protein, which binds to biotin and is obtained from the bacteria *Streptoimyces avidinii*. When compared to Avidin, Streptavidin exhibits less non-specific binding than avidin. The dissociation constant (K_D) of Streotavidin-biotin complex is around 10^{-14} M, which is one of the strongest non-covalent bonds.

G-Biosciences HOOK™ Maleimide Activated Streptavidin is offered to enable its conjugation with fluorescent dyes or ELISA enzymes such as horseradish peroxidase (HRP) containing sulfhydryl (-SH) groups. Streptavidin conjugated with dyes or enzymes is frequently used in DNA hybridization techniques, immunohistochemistry, ELISA and flow cytometry. Biotin conjugated probes or primary antibodies are used to localize antigen and streptavidin conjugated to fluorescent probe or enzyme binds to biotin and detects the antigen. Besides this streptavidin can also be conjugated to antibodies to localize antigen and biotin conjugated to the detection molecules.

Streptavidin is maleimide activated using Sulfo-SMCC, a heterobifunctional crosslinker which adds a free maleimide group, which reacts with sulfhydryl containing molecules.

HOOK™ Maleimide Activated Streptavidin reacts with sulfhydryl containing proteins at pH6.5-7.5 to form stable thioether bonds (Fig.1)

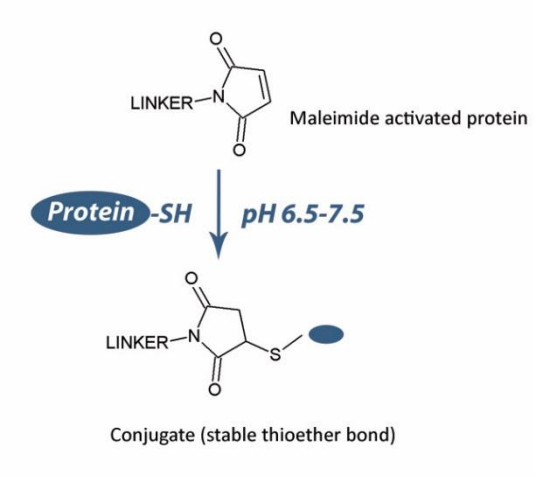


Fig1: Maleimide mediated conjugation reaction

ITEMS SUPPLIED

Cat. #	Description	Size
786-1653	HOOK™ Maleimide Activated Streptavidin	1 mg
786-1654	HOOK™ Maleimide Activated Streptavidin	5 x 1 mg

STORAGE CONDITIONS

HOOK™ Maleimide Activated Streptavidin is supplied at ambient temperature. Upon receipt, store at -20°C under desiccating conditions.

ADDITIONAL ITEMS NEEDED

1. Sulfhydryl group containing protein or peptide that needs to be conjugated.
2. Maleimide conjugation buffer [Optimizer Buffer™ III (5X), Cat. # BKC-06] or 100 mM sodium phosphate, 5-10 mM EDTA, pH7.6.

IMPORTANT INFORMATION

1. Reconstitute the HOOK™ Maleimide Activated Streptavidin immediately before the conjugation reaction. Maleimide groups in solution are hydrolyzed and become non-reactive, no stock solutions for storage should be made. Any left-over solution should be discarded.
2. Sulfhydryl containing compounds should be avoided during conjugation reaction as these will react with maleimide groups and reduce the conjugation efficiency with the desired molecule (Table 1).
3. The conjugation reaction for maleimide activated proteins should be set at pH 6.5 to 7.5, where it forms stable thioether bonds. Maleimide groups can hydrolyze or show reactivity toward primary amines at pH greater than 7.5.
4. In general, 1 mg HOOK™ Maleimide Activated Streptavidin is sufficient to label 1 mg of antibody or protein solution. However, if optimum concentration is necessary for more efficient results, once can optimize protein to HOOK™ Maleimide Activated Streptavidin ratio.

Interfering agents	Recommended
pH	Neutral (6.5-7.5)
Primary amines	Yes
Reducing reagents	NO
Sodium azide	<0.1%

Table 1: *Recommended buffer conditions and components*

PROTOCOL

Preparation of protein for conjugation to maleimide activated protein

1. Proteins or antibodies to be conjugated must have free sulfhydryl groups. Sulfhydryl groups can be introduced to protein using SATA or Traut's Reagent (Cat. # 786-1645, 786-1650). In case of antibodies, disulfide bridges can be cleaved to release free thiols, however, ensure experimentally that affinity of the

antibody is not compromised.

NOTE: *Conjugating molecule can be dye or any other ligand as long as it has free SH group*

2. Protein should be dissolved in maleimide conjugation buffer. If protein is present in buffer with pH > 7.5 or contains reducing agents, it should be dialyzed (Tube-O-DIALYZER™, Cat. #786-610 to 786-624) against the maleimide conjugation buffer or use desalting column (SpinOUT™ GT-600, Cat. # 786-704 or SpinOUT™ G-Acryl 600, 5ml, Cat. # 786-1623) for buffer exchange.

Conjugation reaction

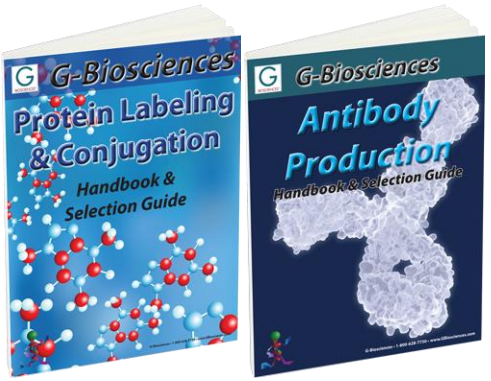
1. Allow the vial of HOOK™ Maleimide Activated Streptavidin to warm to room temperature.
2. Remove the crimp seal and add the protein solution to the lyophilized powder.
3. Dissolve the lyophilized HOOK™ Maleimide Activated Streptavidin in protein solution with the help of a pipette.
4. Incubate the solution for 3-4 hours at room temperature. Alternatively, the conjugation can be set overnight at room temperature.
5. Store the conjugated protein at 4°C.

STORAGE OF CONJUGATED ANTIBODIES/PROTEINS

Store the Streptavidin conjugated antibodies or proteins at 4°C. Conjugates can be stored at -20°C after adding glycerol up to 50% concentration. Optimum storage for a conjugate should be determined by experimentation.

RELATED PRODUCTS

Download our Protein Labeling and Conjugation and Antibody Production Handbooks.



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