HOOK™ Maleimide Activated Allophycocyanin-XL

For conjugation of Allophycocyanin-XL to sulfhydryl groups containing proteins, peptides and ligands

(Cat. #786-1662, 786-1663)

INTRODUCTION

Phycobiliproteins are fluorescent proteins obtained from cyanobacteria and eukaryotic algae. The fluorescence of these proteins is very high, when compared to chemical fluorescent probes, such as fluorescein and rhodamine. Allophycocyanin (APC) is a fluorescent protein isolated from cyanobacteria algae of the Spirulina genus. APC absorbs light at 651 nm and emits red light at 660 nm (Fig.2). APC has high absorptivity and high quantum efficiency. Cross-linked Allophycocyanin (Allophycocyanin-XL) is more stable compared to APC. Allophycocyanin-XL is useful in applications that require fluoroscopy such as fluorescent activated cell sorting, flow cytometry, immunostaining. For this Allophycocyanin-XL needs to be conjugated to antibodies or other molecules and that requires Allophycocyanin-XL to be activated with cross-linker.

G-Biosciences HOOK™ Maleimide Allophycocyanin-XL is offered to enable its conjugation with antibodies or other molecules for its application in immunostaining and flowcytometry.

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

HOOK™ Maleimide Activated Allophycocyanin-XL reacts with sulfhydryl containing proteins at pH6.5-7.5 to form stable thioether bonds (Fig.2)

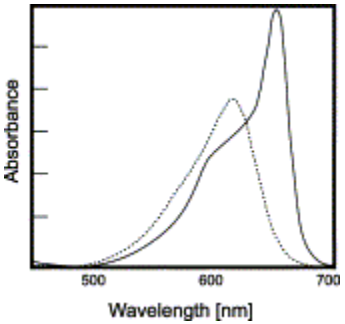


Fig1.: Absorpton and emission spectrum of Allophycocyanin

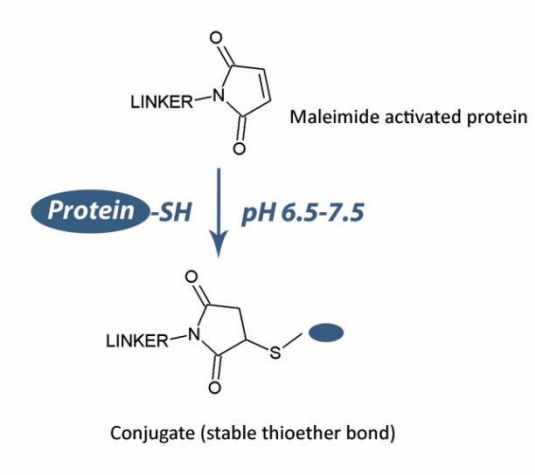


Fig1: Maleimide mediated conjugation reaction

ITEMS SUPPLIED

Cat. #	Description	Size
786-1662	HOOK™ Maleimide Activated Allophycocyanin-XL	1 mg
786-1663	HOOK™ Maleimide Activated Allophycocyanin-XL	5 x 1 mg

STORAGE CONDITIONS

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

ADDITIONAL ITEMS NEEDED

1. Sulfhydryl group containing protein or peptide 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 Allophycocyanin-XL immediately before the conjugation reaction. Maleimide group 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 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 Allophycocyanin-XL 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 Allophycocyanin-XL 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.
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

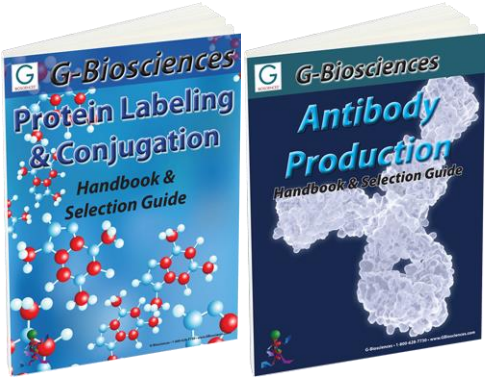
1. Allow the vial of HOOK™ Maleimide Activated Allophycocyanin-XL to warm to room temperature.
2. Remove the crimp seal and add the protein solution to the lyophilized powder.
3. Dissolve the lyophilized HOOK™ Maleimide Activated Allophycocyanin-XL in protein solution with the help of a pipette.
4. Incubate 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 Allophycocyanin-XL 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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