



G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ [technical@GBiosciences.com](mailto:technical@GBiosciences.com)

A Geno Technology, Inc. (USA) brand name

# HOOK™ Maleimide Activated R-Phycoerythrin

For conjugation of R-Phycoerythrin to sulfhydryl  
groups containing proteins, peptides and ligands

(Cat. #786-1657, 786-1658)



think proteins! think G-Biosciences [www.GBiosciences.com](http://www.GBiosciences.com)

INTRODUCTION ..... 3

ITEMS SUPPLIED ..... 4

STORAGE CONDITIONS ..... 4

ADDITIONAL ITEMS NEEDED ..... 4

IMPORTANT INFORMATION ..... 4

PROTOCOL ..... 5

    PREPARATION OF PROTEIN FOR CONJUGATION TO MALEIMIDE ACTIVATED PROTEIN 5

    CONJUGATION REACTION ..... 5

STORAGE OF CONJUGATED ANTIBODIES/PROTEINS ..... 5

RELATED PRODUCTS ..... 6

## 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. R-Phycoerythrin (R-PE) is a phycobiliprotein derived from *Porphyra tenera*, an algae. It has bright red fluorescence at around 578 nm. It is excited from 488-565nm with absorption maximum at 496, 546 and 565 nm (Fig1). R-Phycoerythrin (R-PE) labelled antibodies and other molecules can provide greater sensitivity in immunostaining and flowcytometry compared to chemical fluorescent probes.

G-Biosciences HOOK™ Maleimide Activated R-Phycoerythrin is offered to enable its conjugation with antibodies or other molecules for its application in immunostaining and flowcytometry. R-Phycoerythrin is maleimide activated using Sulfo-SMCC, a heterobifunctional crosslinker, which adds a free maleimide group, which reacts with sulfhydryl containing molecules.

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

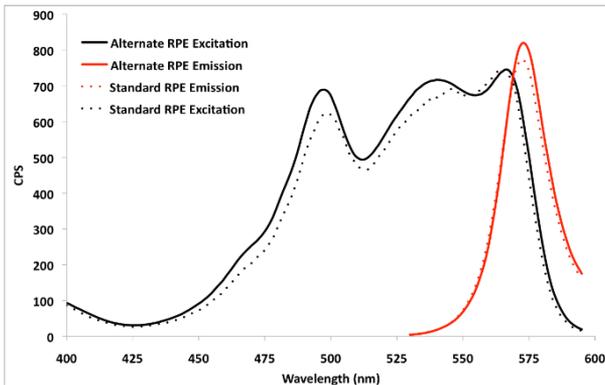


Fig1.: Absorption and emission spectrum of R-Phycoerythrin

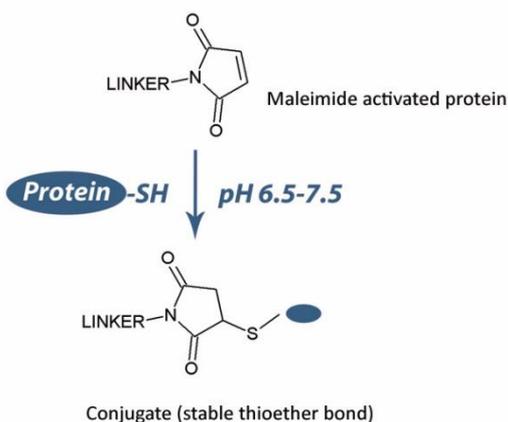


Fig1: Maleimide mediated conjugation reaction

## ITEMS SUPPLIED

Cat. #	Description	Size
786-1657	HOOK™ Maleimide Activated R-Phycoerythrin	1 mg
786-1658	HOOK™ Maleimide Activated R-Phycoerythrin	5 x 1 mg

## STORAGE CONDITIONS

HOOK™ Maleimide Activated R-Phycoerythrin 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 R-Phycoerythrin 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.

- In general, 1 mg HOOK™ Maleimide Activated R-Phycoerythrin 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 R-Phycoerythrin ratio.

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

**Table 1:** Recommended buffer conditions and components

## PROTOCOL

### **Preparation of protein for conjugation to maleimide activated protein**

- 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.
- 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 -786-624) against the maleimide conjugation buffer or use desalting column (Spin-OUT™ GT-600, Cat. # 786-704or SpinOUT™ G-Acryl 600, 5ml, Cat. # 786-1623) for buffer exchange.

### **Conjugation reaction**

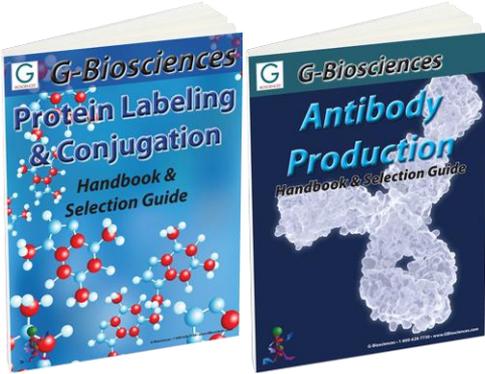
- Allow the vial of HOOK™ Maleimide Activated R-Phycoerythrin to warm to room temperature.
- Remove the crimp seal and add the protein solution to the lyophilized powder.
- Dissolve the lyophilized HOOK™ Maleimide Activated R-Phycoerythrin in protein solution with the help of a pipette.
- Incubate the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

## STORAGE OF CONJUGATED ANTIBODIES/PROTEINS

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

## RELATED PRODUCTS

Download our Protein Labeling and Conjugation and Antibody Production Handbooks.



<http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook>

<http://info.gbiosciences.com/complete-Antibody-Production-handbook>

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.





[www.GBiosciences.com](http://www.GBiosciences.com)