HOOK™ AP SULFO Labeling Kit

A kit for coupling Alkaline AP to proteins via sulfhydryls

INTRODUCTION

The HOOK™ AP SULFO labeling kit is an efficient enzyme labeling kit for tagging proteins with alkaline phosphatase (AP) enzyme. This kit has activated AP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The activated AP saves time as the first step of the normal two steps maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of AP conjugates using free sulfhydryls the kit is supplied with SATA (*N*-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine·HCl, a mild reducing agent for conjugating AP to immunoglobulin G (IgG) and its fragments.

ITEMS INCLUDED Cat #: 786-315

Description	Size
HOOK™ AP SULFO	5x1mg
5X Optimizer Buffer™ III	25ml
SATA	10mg
2-mercaptoethylamine·HCl	50mg
Tube-O-Reactor™ (Medi)	5 Tube-O-Dialyzer™ (8kDa MWCO), with 5 Micro Dialysis Cups, 5 Floats and 30 Glass Balls
DMF	1ml
Hydroxylamine	10mg

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, immediately remove HOOK™ AP SULFO and SATA and store at -20°C protected from moisture. Store the Tube-O-Reactor™ and 2-mercaptoethylamine·HCl at 4°C. Store other components at room temperature. When stored and used properly the kit is stable for one year.

PREPARATION BEFORE USE

Optimizer Buffer™ III: Supplied as a 5X solution. Mix with 4 volumes distilled water. For 100ml, add 20ml Optimizer Buffer™ III to 80ml distilled water.

PROTOCOL

The following protocols are designed for the conjugation of enzyme to immunoglobulin G (IgG) molecules, but they can be adapted for use with other proteins, peptides or ligands.

For Conjugating to IgG

1. Dissolve the HOOK™ AP SULFO in 1ml (1mg/ml) reduced IgG. See Selective Reduction of IgG protocol below.
2. Incubate at room temperature for 1 hour with gently tumbling. Longer incubations, up to 12 hours, may lead to increased conjugation efficiency.

The conjugated antibody is now ready for use. For long-term storage, remove the EDTA by dialysis and add glycerol to a final concentration of 50% and store at -20°C.

For Selective Reduction of IgG

1. Weigh out 1.5mg 2-mercaptoethylamine and add to 1ml of 1mg/ml IgG solution. Dissolve with gentle pipetting. Incubate at 37°C for 90 minutes.

2. The reducing agent can be removed from the IgG solution using the supplied Tube-O-Reactor kit. Transfer the solution to the Tube-O-Dialyzer™ and replace the cap, ensuring that the cap is not over tightened.

3. Fill the dialysis cup with 1X Optimizer Buffer III and add several glass beads to the cup. Using the supplied float invert the Tube-O-Dialyzer™ into the cup and place on an orbital shaker. Gently shake for 1-3 hours with several changes of the conjugation buffer.

NOTE: *Optimizer Buffer™ III contains EDTA to prevent oxidation of the reduced IgG. If another buffer is used ensure it contains 1-10mM EDTA.*

4. After dialysis, use the reduced IgG as soon as possible to prevent reoxidation.

For Addition of Sulfhydryls with SATA

If your protein or peptide lacks free sulfhydryls or have very few, additional free sulfhydryl groups can be added with the use of SATA.

1. Weigh out 2mg SATA into a clean tube and immediately before use dissolve in 200µl DMF.

2. Add 4µl SATA solution to 1ml (1mg/ml) IgG solution to give a 25 molar excess of SATA. Mix and incubate at room temperature for 30 minutes.

3. The modified IgG solution is stable and can be stored at -20°C.

4. Deacetylation is required to complete the reaction. Weigh out 2mg hydroxylamine into a clean tube and immediately before use add 100µl 1X Optimizer Buffer™ III to make the deacetylation solution.

5. Add 50µl deacetylation solution to the IgG solution and incubate for 2 hours at room temperature.

6. The byproducts of the reaction can be removed from the modified IgG by dialysis using the supplied Tube-O-Reactor kit. Transfer the solution to the Tube-O-Dialyzer™ and replace the cap, ensuring that the cap is not over tightened.

7. Fill the dialysis cup with 1X Optimizer Buffer III and add several glass beads to the cup. Using the supplied float invert the Tube-O-Dialyzer™ into the cup and place on an orbital shaker. Gently shake for 1-3 hours with several changes of the conjugation buffer.

8. After dialysis, use the modified IgG as soon as possible.

RELATED PRODUCTS:

1. HOOK™ HRP SULFO Labeling Kit (Cat. # 786-314) *for labeling proteins with horseradish peroxidase enzyme through sulfhydryl groups.*

2. HOOK™ HRP PLUS Labeling Kit (Cat. # 786-313) *for labeling proteins with horseradish peroxidase enzyme through amine groups.*

3. Tube-O-Dialyzer™ *mini dialysis units for removal of unwanted reagents and byproducts. Available with MWCO limit 1, 4, 8, 15 and 50kDa.*

4. Spin-OUT™ columns *for rapid processing and desalting of enzyme conjugated proteins.*

Note: For other related products, please visit our web site at www.GBiosciences.com

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