HOOK™ HRP PLUS Labeling

Coupling activated HRP (periodate-treated, aldehyde-activated) to Proteins, via Amines

(Cat. # 786-1652, 786-1664)

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INTRODUCTION

The HOOK™ HRP PLUS labeling kit is a high efficiency horseradish peroxidase (HRP) enzyme labeling kit for tagging the enzyme with proteins. The HRP is activated by treating it with metaperiodate which produces the aldehyde groups in carbohydrate moiety of HRP. This activated HRP (HRP PLUS) couples with high efficiency (>90%) to the numerous amine groups of proteins and is superior to the commonly used glutaraldehyde coupling chemistry.

HOOK™ HRP PLUS labeling kit contains reagents for carrying out five conjugation reactions each of 1 mg antibody with HRP.

ITEM(S) SUPPLIED

Description	Cat. # 786-1664	Cat. # 786-1652
HOOK™ HRP PLUS	5 x 1mg	5 x 1mg
Sodium Cyanoborohydride [5M]	0.5ml	-
Quenching Buffer	25ml	-
JAW™ PBS Buffer Pack (1L)	1 Pack	-
JAW™ Carbonate / Bicarbonate Buffer Pack (1L)	1 Pack	-
SpinOUT™ GT-600, 5 ml	5 columns	

STORAGE CONDITION

The kit is shipped at ambient temperature. On arrival, immediately remove HOOK™ HRP PLUS and store at -20°C protected from moisture. Store other components at 4°C.

ADDITIONAL ITEMS REQUIRED

Protein or antibody to be conjugated.

IMPORTANT INFORMATION

- No amine-containing buffers, Tris or glycine, should be used. If proteins are in amine-containing buffers then dialyze the samples against conjugation buffer to completely remove the amines.
- *Conjugation Buffer*: The supplied Carbonate/Bicarbonate buffer or PBS buffer provide the ideal conditions for conjugation. The pH of the reaction can be controlled to affect the efficiency of the coupling of the enzyme to a protein and therefore the size of the resultant protein-enzyme complexes formed. At more alkaline pH, provided by the Carbonate/Bicarbonate buffer (pH9.4), Schiff's base formation occurs rapidly and with high efficiency, resulting in conjugates with greater HRP incorporation and higher molecular weight. At physiological pH, provided by the PBS buffer (pH7.4), initial Schiff's base formation is slower

resulting in lower coupling efficiency and lower molecular weight conjugates. Coupling at physiological pH is recommended for immunohistochemical staining and blotting techniques as the complex will penetrate easier and washing steps will be more efficient resulting in lower backgrounds. The alkaline pH coupling is recommended for ELISA procedures, where higher sensitivity is important, but washing off excess conjugate is not a problem.

- ***WARNING:*** *Sodium cyanoborohydride is toxic, open tubes and prepare solutions in a fume hood.*

PREPARATION BEFORE USE

- Dissolve the JAW™ Buffer Packs in 1L DI water each. These are the conjugation buffers.
- *IgG (Protein) Preparation*: Prepare a 1mg/ml of IgG in conjugation buffer to give approximately a 4-fold molar excess of HRP to IgG.

PROTOCOL

Conjugation of protein or antibody with HRP

1. Dissolve 1mg of protein to be coupled in 1ml conjugation buffer.
2. Add the protein solution to the vial of HOOK™ HRP PLUS and gently pipette up and down to reconstitute the activated enzyme.
   NOTE: *The 1:1 ratio of protein: HRP will result in a 3.75 molar excess of HRP over the amount of IgG. For conjugates consisting of greater enzyme-to-antibody ratios, proportionally increase the amount of enzyme solution as required. Typically, molar ratios of 4:1 to 15:1 (enzyme: antibody) give acceptable conjugates.*
3. **In a fume hood:** If using PBS as the conjugation buffer, immediately add 10µl sodium cyanoborohydride. If using the carbonate/bicarbonate buffer, do not add at this stage.
4. Incubate at room temperature for one hour with gentle shaking or tumbling.
5. If using PBS, go to the next step. If using carbonate/bicarbonate buffer, ***in a fume hood*** add 20µl sodium cyanoborohydride [5M] and incubate at room temperature for 15 minutes.
6. Add 50µl quenching buffer and incubate with gentle tumbling or shaking for 15 minutes.
7. Remove the excess sodium cyanoborohydride using SpinOUT™ GT-600, 5 ml column.

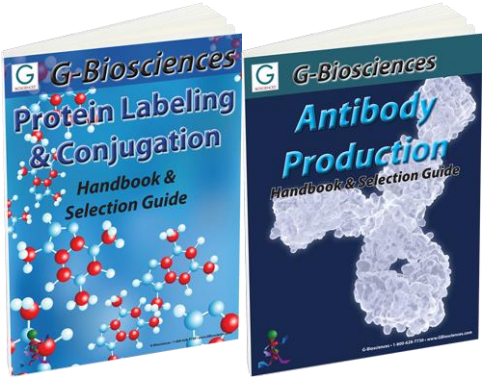
Removal of sodium cyanoborohydride using SpinOUT™ GT-600, 5ml column

1. Centrifuge the SpinOUT™ GT-600 column at 1,000g for 2 minutes to compact the resin.
2. Prepare the Spin-*OUT*™ GT-600 column by removing the top and then bottom caps. Place into an appropriate collection tube.
3. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
4. Place the column in a new collection tube and remove the cap.

5. Add 10 ml of PBS to the center of column.
6. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
7. Repeat steps 6 and 7 two more times, ensuring the buffer is discarded after each centrifugation.
8. Centrifuge the column at 1,000 g for 2 minutes to remove residual buffer
9. Place the column in a new collection tube and remove the cap.
10. Slowly, apply 1 ml of HRP conjugated antibody solution to the center of column.
11. Centrifuge the column at 1,000g for 4 minutes to collect the conjugated antibody solution free of sodium cyanoborohydride.
12. For long-term storage, add glycerol to a concentration of 50% and store at -20°C.

RELATED PRODUCTS

Download our Protein Labeling and Conjugation and Antibody Production Handbooks.



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