HOOK™ Agarose Coupling Kit (*Amine Reactive*)

For Agarose Affinity columns using your Proteins or Peptides

INTRODUCTION

The amine reactive HOOK™ Agarose Coupling Kit is designed for the simple and efficient coupling of proteins and peptides to a solid agarose support.

This kit utilizes 6% agarose that has been activated to generate reactive aldehyde groups. The aldehyde groups of the agarose react spontaneously with primary amines, located at the N-terminus of proteins or in lysine residues, to form intermediate Schiff Base complexes. These, in turn, are selectively reduced by reductive amination to form stable amine linkages between the agarose and the ligand.

This kit allows for the generation of five stable and reusable columns (4ml slurry/column) for the affinity purification of antibodies, proteins and other protein interacting biomolecules.

ITEM(S) SUPPLIED **Cat. # 786-063**

Description	**Size**
5X Optimizer Buffer™ VI	2 x 25ml
Sodium Cyanoborohydride ($NaCNBH_3$)	1 vial (0.4g)
HOOK™ Activated Agarose slurry (Amine reactive)	20ml
Empty Disposable Columns *(Part# 069E-B)*	5
Quenching Buffer (3M)	25ml

STORAGE CONDITION

The kit is shipped at ambient temperature. On arrival store 5X Optimizer Buffer™ VI and HOOK™ Activated Agarose slurry (Amine reactive) at 4°C, Sodium Cyanoborohydride ($NaCNBH_3$) desiccated at Room Temp (RT) and all other components at RT. When stored and used properly the kit is stable for one year.

ITEMS NEEDED BUT NOT SUPPLIED WITH KIT

NaOH (25mM), Phosphate Buffer Saline (PBS), and Sodium Azide.

PREPARATION BEFORE USE

Prepare 1X Optimizer Buffer™ VI (1ml 5X Optimizer Buffer™ VI in 4ml de-ionized water). The standard protocol uses 35ml 1X Optimizer Buffer™ VI.

***CAUTION**: No amine-containing buffers, Tris or glycine, should be used. If proteins are in amine-containing buffers, then dialyze the samples against 1X Optimizer Buffer™ VI to completely remove the amines. For easy and convenient dialysis, use G-Biosciences' Tube-O-Dialyzer™ (supplied separately), visit www.GBiosciences.com .*

CAUTION:** The pH of all buffers used should be greater than pH 7.0 to prevent the release of toxic gas.* ***DO NOT ACIDIFY SOLUTIONS.

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

PROTOCOL

NOTE: *To ensure complete coupling, we advise using the recommended quantities of reagents, as these provide a molar excess of protein or peptide to the reactive groups on the agarose. Ensure complete and thorough mixing during shaking of the agarose to prevent beads from drying out.*

1) Allow the reagents to reach room temperature.

2) Gently resuspend the HOOK™ Agarose by inverting the bottle 2-3 times. Once a homogeneous slurry is formed, remove 4ml of the slurry and place in a 15ml tube. Centrifuge at 2000xg for 30-60 seconds. Remove the storage buffer with a pipette.

NOTE: *The binding capacity of the agarose is ~20mg protein/ml resin.*

3) Equilibration Step: Add 10ml 1X Optimizer Buffer™ VI to the HOOK™ Agarose and place on a rocker for 3-5 minutes. Centrifuge at 2000xg for 30-60 seconds. Remove the buffer with a pipette. Repeat Step 3 once.

4) Protein/Peptide Solution: Make 2ml solutions of either 1-20mg/ml protein or 1-2mg/ml peptide in 1X Optimizer Buffer™ VI, H_2O or phosphate buffer saline. Add the protein/peptide solution and an additional 3ml 1X Optimizer Buffer™ VI to the HOOK™ agarose from step 3.

NOTE: *For peptides insoluble in aqueous solutions, we recommend the use of DMSO (<30%).*

OPTIONAL: Remove an aliquot of the protein solution to be used as 'starting material' to determine coupling efficiency, if required.

CAUTION: THE FOLLOWNG STEP-5 AND 6 MUST BE PERFORMED IN A FUME HOOD

5) Reductant Solution Preparation: Weigh out 50-100mg Sodium Cyanoborohydride. Add 6 µl NaOH (25mM) for each mg of Sodium Cyanoborohydride. Vortex until you have a clear solution.

6) Add 200µl Reductant Solution to the HOOK™ Agarose/protein/peptide solution from step 4. Mix with gentle shaking for 6 hours.
After this step, the reaction may be removed from the hood. Further incubation may be carried out at 4°C overnight.

7) Centrifuge at 2000xg for 30-60 seconds. Remove the supernatant with a pipette and save for testing coupling efficiency or discard.

NOTE*: The coupling efficiency is determined by measuring and comparing the concentration of the protein/peptide in 'starting material' (Step 4) and in the supernatant (Step 7). The concentration may be determined either by UV absorbance at 280nm or by protein assay (CB-X assay, Cat # 786-12X).*

8) Wash the coupled agarose twice with 5ml of 1X Optimizer Buffer™ VI as in Step 3.

9) Blocking Step: Add 5ml Quenching Buffer to the agarose and gently shake for one hour at room temperature.

10) Centrifuge at 2000xg for 30-60 seconds and remove the buffer with a pipette. Wash twice with 5ml PBS or buffer of choice as described in Step 3.

11) Transfer the coupled agarose to the column and store at 4°C in PBS or buffer of choice with 0.05% sodium azide. The column is now ready for use.

RELATED PRODUCTS

1. HOOK™ Agarose Coupling Kit (Sulfhydryl reactive) (Cat.# 786-064): *for the coupling of peptides and proteins to agarose through their sulfhydryl groups.*

2. HOOK™ Agarose (Amine reactive) (Cat.# 786-066): *extra agarose for the coupling of peptides and proteins via their primary amine residues.*

3. Empty columns (Cat.# 786-169): *extra empty columns for the generation of small affinity columns.*

4. HOOK™-Biotin Kit: *supplied with functional biotin agent and a complete set of accessories and buffers for easy labeling.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*