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A Geno Technology, Inc. (USA) brand name

HOOK™ Activated Agarose (Amine Reactive)

(Cat. # 786-066)

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INTRODUCTION

The amine reactive HOOK™ Activated Agarose is 6% agarose that has been activated to generate reactive aldehyde groups.

The resin consists of 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 by sodium cyanoborohydride to form stable amine linkages between the agarose and the ligand.

ITEM(S) SUPPLIED (Cat. #: 786-066)

Description	Size
HOOK™ Activated Agarose (Amine Reactive)	20ml slurry

STORAGE CONDITION

It is shipped at ambient temperature. Upon receiving store at 4°C

BINDING CAPACITY

- ~20mg protein/ml resin

ADDITIONAL ITEMS REQUIRED ITEMS

- Phosphate Buffer Saline (PBS)
- 5M Sodium Cyanoborohydride in 1M NaOH
- 1M Tris-HCl, pH7.4
- 1M Sodium Chloride
- Suitable Disposable Column
- Sodium Azide.

IMPORTANT INFORMATION

CAUTION*: No amine-containing buffers, Tris or glycine, should be used. If proteins are in amine-containing buffers, then dialyze the samples against 1X PBS to completely remove the amines. For easy and convenient dialysis, use G-Biosciences' Tube-O-Dialyzer™.*
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 an homogeneous slurry is formed, remove an appropriate volume of the slurry and place in a suitable size 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 5 resin volumes (RV) 1X PBS 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 solutions of either 1-20mg/ml protein or 1-2mg/ml peptide in H_2O or phosphate buffer saline. Add the protein/peptide solution to the equilibrated resin. Do not exceed the binding capacity of the resin. We recommend adjusting the final volume to 2.5 resin volumes, for 2ml resin use a final volume of 5ml, adjust volumes with 1X PBS.
   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 MUST BE PERFORMED IN A FUME HOOD
5. Add 10µl 5M Sodium Cyanoborohydride in 1M NaOH for every 1ml of protein/peptide solution added to the HOOK™ Agarose from step 4 to give ~50mM Sodium Cyanoborohydride. Mix with gentle shaking for 6 hours.
6. 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 with 5 resin volumes of 1X PBS and repeat once as in Step 3.
9. Blocking Step: Add 2.5 resin volumes of 1M Tris-HCl, pH7.4 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.

APPENDIX: GENERAL PROTOCOL FOR AFFINITY PURIFICATION OF PROTEIN

Sample Preparation

Dissolve 1-20mg protein or 1-2mg peptide to be immobilized in 2-3mL of 1X PBS or other suitable buffer.

For proteins already in solution either:

- Dilute sample 4-fold in 1X PBS or other suitable buffer
- Desalt or dialyze to buffer-exchange into1X PBS or other suitable buffer

NOTE: *If the protein solution contains primary amines (e.g., Tris or glycine), these compounds must be thoroughly removed or they will compete with the intended protein-coupling reaction.*

Additional Materials Required

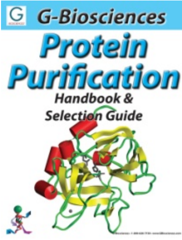
- Binding/Wash Buffer: Phosphate Buffered Saline (PBS), Tris Buffered Saline (TBS) or other suitable buffer.
- Sample: See sample preparation above
- Elution Buffer: 0.1-0.2M glycine•HCl, pH 2.5-3.0
- Neutralization Buffer: 1M phosphate or 1M Tris; pH 9

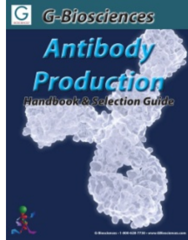
Protocol

1. Allow the affinity column to equilibrate to room temperature and allow the storage buffer to drain out.
2. Equilibrate column by adding 3 column volumes (CV) Binding/Wash Buffer and allowing it to drain from column.
3. Add Sample to column and allow it to flow into the resin bed. For samples <1CV, we recommend sealing the bottom of the column after the sample as entered the resin and incubating for up to 1 hour. For samples >1CV, seal the column after the addition of each CV and incubate to maximize binding.
4. Unseal the column and wash the column with 6CV of Binding/Wash Buffer.
5. Elute the bound protein by applying 4CV of Elution Buffer. Collect 0.5-1ml fractions. Monitor elution by absorbance at 280nm or a suitable protein assay. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.
6. The pH of each fraction can be adjusted to neutral by adding 50µl of Neutralization Buffer per 1ml of eluate.
7. Monitor elution by absorbance at 280nm. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.

RELATED PRODUCTS

Download our Protein Purification or Antibody Production Handbooks.





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