G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

NHS-Activated Agarose (Dry Form)

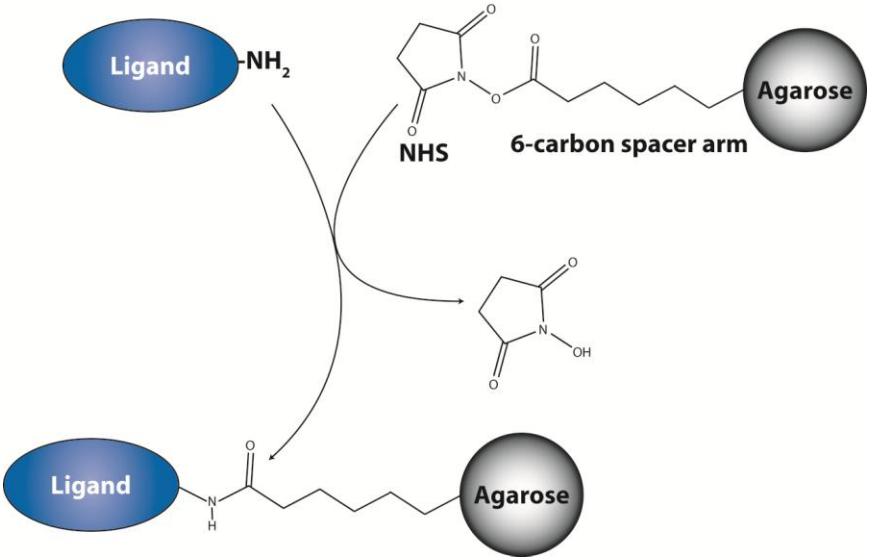
For covalent binding of primary amine containing ligands to a solid support

(Cat. #786-1211, 786-1212, 786-1213, 786-1214)

INTRODUCTION

NHS-Activated Agarose consists of 6% cross-linked agarose that has been activated by the addition of a reactive NHS (N-hydroxysuccinimide) group. The NHS group forms covalent, chemically stable amide bonds with ligands that contain primary amines. The NHS-Activated Agarose also contains a spacer arm between the NHS group and the agarose beads, making it suitable for coupling of small proteins and peptides. The long spacer arm minimizes steric hindrance allowing high efficient binding of ligands, including small proteins and peptides.

The 6% highly cross-linked agarose beads are coupled to 6-carbon spacer arm. The terminal carboxyl group of the spacer arm is activated by esterification with the NHS group.



The coupling reaction is performed in an amine-free buffer at pH7-9 and the coupling efficiency is typically >80%, regardless of ligand's pI or molecular weight. Once the ligand is coupled to the resin, the resin can be used for multiple affinity purifications. The resin is suitable for gravity-flow and low- to medium-pressure applications.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1211	NHS-Activated Agarose (Dry Form)	1 g
786-1212	NHS-Activated Agarose (Dry Form)	5 g
786-1213	NHS-Activated Agarose (Dry Form) with Spin Columns	1g with 5 x1 ml Spin Columns
786-1214	NHS-Activated Agarose (Dry Form) with Spin Columns	5 g with 5 x3 ml Spin Columns

STORAGE CONDITION

Supplied as a dry form to maintain activity. It is shipped at ambient temperature. Upon receiving store at 4°C

PROPERTIES

- 90µm mean particle size
- 45-165µm particle size range
- Spherical, highly cross-linked 6% agarose
- Hydrated resin volume:200 mg resin hydrates and swells to ~ 0.5 ml
- Resin coupling capacity is 2-20 mg or 1-2 mg peptide or other small molecules per 1 ml fully swelled resin

ADDITIONAL ITEMS REQUIRED

- Empty columns (Cat. # 786-810, 786-724)
- Coupling/Wash Buffer: 0.2M $NaHCO_3$, 0.5M NaCl, pH8.3 or other amine free buffer at pH7-9.
- Ligand, Protein or Peptide with primary amines
- Quenching Buffer: 1M ethanolamine or 1 M Tris, pH7.5

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™.*

PREPARATION

1. Allow the dry NHS-resin to reach room temperature.
2. For insoluble, hydrophobic ligands, supplement Coupling/Wash Buffer with 20% DMSO or 4M Guanidine•HCl
3. Remove an aliquot of the protein solution to be used as 'starting material' to determine coupling efficiency, if required.
4. 80% of the reaction occurs in the first 30 minutes. The reaction can be extended to 4 hours at room temperature and incubations overnight can be performed at 4°C.

5. Remove the caps from the column and transfer the column to a suitable collection tube. Centrifuge at 1,000g for1 minute. Save the flow-through to assay binding efficiency.
   ***NOTE**: The coupling efficiency is determined by measuring and comparing the concentration of the protein/peptide in 'starting material' and in the supernatant. The concentration cannot be determined either by UV absorbance at 280nm or BCA Protein assays due to the presence of the NHS leaving group. The protein concentration can be determined with RED660 protein assay (Cat. # 786-676) or CB-X™ protein assay (Cat. # 786-12X). Alternatively, remove the NHS leaving group by dialysis using our Tube-O-Dialyzer (Cat. # 786-610-786-624).*
6. Wash the resin with 1CV of Coupling/Wash Buffer. Centrifuge at 1,000g for 1 minute. Repeat once.
7. Blocking Step: Add 1CV Quenching Buffer to the resin and seal the column.
8. Incubate at room temperature for 15 minutes with end-over-end mixing.
9. Remove the top then bottom cap and centrifuge the column at 1,000g for 1 minute. Discard the flow-through.
10. Wash column with at least 3CV Coupling/Wash Buffer. Monitor washes for presence of protein to ensure all unbound protein has been washed away.
11. Use the column for affinity purifications or wash the column with 3 CV PBS with 0.05% sodium azide or other preservative. Seal the column with 0.5-1ml buffer remains above the column and store upright at 4°C.

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.

Column Regeneration

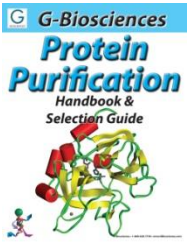
1. Wash the column with 4CV Binding/Wash Buffer.
2. Equilibrate column with 2CV Binding/Wash Buffer with 0.05% sodium azide.
3. Seal column with 1CV Binding/Wash Buffer with 0.05% sodium azide. Store upright at 4°C.

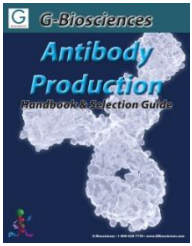
Troubleshooting

Issue	**Possible Cause**	**Solution**
Low coupling efficiency	Interferences from other primary amines, possibly from amines in buffers.	Dialyze or desalt sample to ensure all amine containing buffers are removed
Protein or peptide is insoluble in Coupling/Wash Buffer	Hydrophobic molecule	Supplement Coupling/Wash Buffer with 20% DMSO or 4M Guanidine•HCl
Loss of binding capacity of affinity column overtime	The coupled ligand has been damaged over time	Prepare new column
	Non-specific interactions blocking binding capacity	Was column in high salt (1M NaCl)

RELATED PRODUCTS

Download our Protein Purification or Antibody Production Handbooks.





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