INSTRUCTIONS

Pierce NHS-Activated Agarose Dry Resin

26196 26197 26198 26199 2221.3

Number	Description
26196	**Pierce NHS-Activated Agarose Dry Resin,** 1g
26197	**Pierce NHS-Activated Agarose Dry Resin,** 5g
26198	**Pierce NHS-Activated Agarose Spin Columns,** 33mg, 25 columns
26199	**Pierce NHS-Activated Agarose Spin Columns,** 330mg, 5 columns
	Support: 6% crosslinked beaded agarose
	Swell volume: 6-7.5mL/g of dry resin
	Storage: Upon receipt store dry resin at -20°C. Product is shipped in an ice pack.

Introduction

The Thermo Scientific™ Pierce™ NHS-Activated Agarose Resin allows simple and efficient covalent immobilization of proteins to a beaded-agarose support, providing a valuable tool for affinity purification of antibodies, antigens or other biomolecules. The activated agarose contains *N*-hydroxysuccinimide (NHS) ester functional groups that react with primary amines on proteins or other molecules to form stable amide linkages. The coupling reaction is performed in an amine-free buffer at pH 7-9. Coupling efficiency is typically greater than 80%, regardless of the ligand's molecular weight or pI. Once the ligand is immobilized, the prepared resin can be used for multiple affinity purification procedures. The crosslinked beaded agarose has fast linear flow potential, making it useful for gravity-flow and low- to medium-pressure applications.

Procedure for Coupling Proteins

A. Additional Materials Required

- Coupling/Wash Buffer: 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 (PBS, Product No. 28372) or other amine-free buffer at pH 7-9. Avoid amine-containing buffers such as Tris or glycine.
- Protein or peptide: The coupling capacity is 1-20mg protein or 1-2mg peptide per mL swelled (hydrated) resin, and 150mg of dry resin will yield approx. 1mL of hydrated resin. Prepare protein or peptide solution in Coupling/Wash Buffer for direct addition to the desired amount of dry resin. (To allow for complete hydration use at least twice the minimum sample volume needed to swell the resin, e.g., prepare 2mL of sample to hydrate 150mg of dry resin.)
- Quenching Buffer: 1M ethanolamine or 1M Tris, pH 7.4
- Spin columns (e.g., Thermo Scientific Pierce Spin Columns, Product No. 69705) and collection tubes (e.g., 2mL Thermo Scientific Pierce Centrifuge Column, Product No. 89896). If using the Pierce NHS-Activated Agarose Spin Columns, use 2mL collection tubes for the 33mg column and 15mL conical tubes for the 330mg column.

B. Protein Immobilization

Note: Add the protein or peptide sample directly to the dry resin. Use at least twice the sample volume to swell volume. The resin swell volume is 6-7.5mL of wet resin per gram of dry resin. For the 33mg spin column, use 400µL of sample; for the 330mg spin column, use 4mL of sample. Also use twice the buffer volume to resin volume in the steps below.

1. Place an appropriate amount of dry NHS-Activated Agarose resin to an empty spin column.
2. Add protein solution to the column. Save 0.1mL of the solution for subsequent determination of coupling efficiency.
3. Replace the top cap on the column and mix the reaction end-over-end for 1 hour.

   Note: Approximately 80% of the reaction occurs in the first 30 minutes. The reaction may be extended to 2 hours at room temperature or overnight at 4ºC.

4. Remove top and bottom caps and place the column in a collection tube. Centrifuge at 1000 × *g* for 1 minute and save the flow-through.
5. Add Coupling/Wash Buffer (twice the volume of the resin) to the column, centrifuge at 1000 × *g* for 1 minute and save the wash fraction. Repeat this step once.

   Note: The saved flow-through and washes can be used to determine the coupling efficiency by comparing the protein concentrations of these non-bound fractions to the starting sample (saved in Step 2). The NHS leaving group interferes with the BCA protein assay and absorbance measurements at 280nm; for optimal results use the Thermo Scientific Pierce 660nm Protein Assay (Product No. 22660) or dialyze the flow-through and wash fractions against PBS using a 20K MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette (Product No. 66003) before measuring the absorbance.

C. Block Remaining Active Sites

1. Add Quenching Buffer (twice the volume of the resin) to the column and replace the bottom cap.
2. Replace the top cap and mix end-over-end for 15-20 minutes at room temperature.
3. Remove the top cap and then the bottom cap. Place the column in a new collection tube, centrifuge at 1000 × *g* for 1 minute and discard the flow-through.
4. Wash column with Coupling/Wash Buffer. Monitor the final washes for the presence of protein by measuring absorbance at 280nm or by the Pierce 660nm Protein Assay. Either proceed directly with affinity purification or prepare column for storage (Step 5).
5. For storage, wash column with PBS containing 0.05% sodium azide or other preservative. Replace bottom cap when 0.5-1mL of buffer remains above the resin bed. Replace top cap and store column upright at 4°C.

General Protocol for Affinity Purification of Protein

Note: This protocol uses a column with a resin-bed volume of 1mL. For columns with other bed volumes, adjust all solution (e.g., sample, wash, and elution) volumes accordingly. The amount of protein sample needed and incubation time are dependent upon the affinity system involved (e.g., antibody-antigen interaction) and must be optimized.

A. Additional Materials Required

- Binding/Wash Buffer: Phosphate-buffered saline (PBS, Product No. 28372), Tris-buffered saline (TBS, Product No. 28379) or other buffer that is compatible with the intended affinity interaction
- Sample: Prepare antigen or other molecule in Binding/Wash Buffer or dilute sample 1:1 in Binding/Wash Buffer
- Elution Buffer: Thermo Scientific IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl, pH 2.5-3.0
- Neutralization Buffer (optional): 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris; pH 9

B. Procedure

Note: Throughout the procedure, do not allow the resin bed to become dry; replace bottom cap as soon as buffer drains down to the top of resin bed.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove top and bottom caps and place column in a collection tube. Centrifuge the column at 1000 × *g* for 2 minutes to remove the storage solution.
3. Equilibrate column by adding 1mL of Binding/Wash Buffer and centrifuge at 1000 × *g* for 2 minutes. Discard buffer from collection tube. Repeat this step twice.
4. Add sample to column and allow it to enter the resin bed. Replace top and bottom caps on the column.
5. Incubate the column. Optimal reaction time depends on the specific affinity interaction. Typically, 2 hours at room temperature with end-over-end mixing is sufficient, or the incubation can be extended to overnight at 4ºC.
6. Remove top cap and bottom caps from column and place column in new collection tube. Centrifuge the column at 1000 × *g* for 2 minutes. Save the flow-through to analyze binding efficiency.
7. Wash the column with 3mL of Binding/Wash Buffer. Centrifuge at 1000 × *g* for 2 minutes.

8. Elute the bound protein by applying 8mL of Elution Buffer. Collect 1mL (or 0.5mL) fractions. The pH of each fraction can be adjusted to neutral by adding 50µL of Neutralization Buffer per 1mL of collected eluate.
9. Monitor elution using the Pierce 660nm Protein Assay. Alternatively, measure the absorbance at 280nm of the eluted fractions. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.

C. Column Regeneration and Storage

Note: Regenerate the column immediately after elution to prevent damage to the immobilized molecule by the low-pH elution buffer.

1. Wash column with 8mL of Binding/Wash Buffer to remove any residual protein and neutralize the elution buffer.
2. Equilibrate column with 4mL of Binding/Wash Buffer containing 0.05% sodium azide.
3. Replace bottom cap and add 2mL of Binding/Wash Buffer to the column and cap the top. Store column upright at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Low coupling efficiency	Primary amine-containing buffer was not completely removed before coupling	Dialyze or desalt sample to completely remove Tris or glycine
Protein is not soluble in Coupling Buffer	Molecule was hydrophobic	Dissolve molecule in Coupling Buffer containing up to 4M guanidine•HCl or 20% DMSO
Affinity column has reduced binding capacity with time	Immobilized sample was damaged by time, temperature or elution conditions	Prepare a new affinity column
	Nonspecifically bound material had reduced capacity	Wash column with high salt (~1M NaCl) to remove nonspecifically bound material

Additional Information Available from our Website

- Tech Tip #12: Prepare molecules with poor solubility for immobilization on affinity supports
- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas solutions for use in affinity and gel filtration columns

Related Thermo Scientific Products

89868 **Pierce Centrifuge Columns,** 0.8mL, 50 centrifuge columns and 50 screw caps

89896 **Pierce Centrifuge Columns, 2mL (resin bed capacity),** gravity or centrifuge compatible, 25 units

89897 **Pierce Centrifuge Columns, 5mL (resin bed capacity),** gravity or centrifuge compatible, 25 units

89898 **Pierce Centrifuge Columns, 10mL (resin bed capacity),** gravity or centrifuge compatible, 25 units

28372 **BupH™ Phosphate Buffered Saline,** each dry-blend pack makes 500mL with water, 40 packs

21004 **IgG Elution Buffer,** low-pH elution buffer for general protein affinity purifications, 1L

22660 **Pierce 660nm Protein Assay**

26200 **Pierce NHS-Activated Agarose Slurry,** 25mL settled resin supplied in acetone

General References

Domen, P., *et al.* (1990). Site-directed immobilization of proteins. *J Chromatogr* **510:**293-302.

Hermanson, G.T., *et al.* (1992). *Immobilized Affinity Ligand Techniques.* Academic Press, Inc.: San Diego, CA.

Lasch, J. and Koelsch, R. (1978). Enzyme leakage and multipoint attachment of agarose-bound enzyme preparations. *Eur J Biochem* **82:**181-6.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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