INSTRUCTIONS

AminoLink® Plus Coupling Resin

20501 20505

0490.5

Number	Description
20501	**AminoLink Plus Coupling Resin**, 10mL resin, 20mL slurry
20505	**AminoLink Plus Coupling Resin**, 50mL resin, 100mL slurry
	Support: 4% crosslinked beaded agarose
	Supplied as 50% slurry in 0.02% sodium azide
	Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific AminoLink Plus Coupling 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 support contains aldehyde functional groups that spontaneously react with primary amines on proteins or other molecules. The Schiff base bonds that form are reduced to stable secondary amine bonds in the presence of the mild reducing agent, sodium cyanoborohydride. Coupling efficiency by this reductive amination mechanism is typically greater than 80%, regardless of the ligand's molecular weight or pI. The resin is crosslinked beaded agarose with fast linear flow potential, making it useful for gravity flow and low- to medium-pressure applications.

Two coupling protocols (pH 7.2 and pH 10) are offered for use with AminoLink Plus Coupling Resin. The pH 10 coupling protocol typically provides greater immobilization yields and ligand densities than the pH 7.2 protocol. The standard, 7.2 pH protocol is offered as an alternative for proteins that are sensitive to the pH 10 environment required for the enhanced coupling protocol. Once the ligand is immobilized, the prepared resin can be used for multiple rounds of affinity purification.

Material Preparation

A. Additional Materials Required

- Column containing the desired slurry volume of AminoLink Plus Coupling Resin

  Note: The following coupling procedure is for 2mL of AminoLink Plus Resin (4mL resin slurry) in a 5mL gravity-flow column (See Related Thermo Scientific Products). Scale procedure as needed. To dispense the resin, invert bottle of slurry several times and then pipette the desired volume into an empty column using a wide orifice or cut pipette tip. For a centrifuge column procedure, see instructions for Product No. 44894.
- Coupling Buffer: 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 (PBS, Product No. 28372), or 0.1M sodium citrate, 0.05M sodium carbonate, pH 10

  Note: Although the entire coupling reaction is effective over a wide pH range, research has demonstrated that maximum protein coupling occurs if Schiff base formation is performed at pH 10, followed by sodium cyanoborohydride reduction at near-neutral pH. Therefore, two coupling protocols (pH 7.2 and pH 10) are provided. Use the simpler pH 7.2 protocol if the protein is known to be unstable or insoluble in the pH 10 environment required for the enhanced coupling protocol.
- Cyanoborohydride Solution ($NaCNBH_3$, Product No. 44892): 5M $NaCNBH_3$ in 1M NaOH

  Note: Prepare this solution in a fume hood because $NaCNBH_3$ is toxic.
- Quenching Buffer: 1M Tris•HCl, pH 7.4
- Wash Solution: 1M sodium chloride (NaCl)

B. Sample Preparation (Protein Solution)

Dissolve 1-20mg protein or 1-2mg peptide to be immobilized in 2-3mL of Coupling Buffer (choose pH 7.2 or pH 10, see Important Product Information). For proteins already in solution, dilute sample 4-fold in Coupling Buffer; alternatively, desalt or dialyze to buffer-exchange into Coupling 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.

Procedure for Coupling Protein Using the pH 10 Coupling Buffer

A. Protein Immobilization

1. Equilibrate upright column containing desired amount of AminoLink Plus Resin to room temperature and allow the resin to settle. Open column and drain storage solution into a collection tube. Throughout entire procedure, do not allow the resin bed to become dry. Place bottom cap on the column when the buffer drains down to the top of the resin bed.
2. Equilibrate column by adding 6mL (3 resin-bed volumes) of pH 10 Coupling Buffer and allowing the contents to drain.
3. Replace the bottom cap and add 2-4mL of the protein solution (dissolved in pH 10 Coupling Buffer) to the column. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.
4. Place the top cap on the column and mix the reaction slurry by gentle end-over-end rocking for 4 hours.

   Note: For proteins that are sensitive to long-term agitation (e.g., precipitate), mix for 2 hours and then allow the column to remain stationary for an additional 2 hours. Longer incubation times are acceptable, depending on protein stability.
5. Remove top and bottom caps and drain the contents of the column into a new collection tube.
6. Save the flow-through and determine the coupling efficiency while continuing with column blocking steps. Determine coupling efficiency by comparing the protein concentrations of the non-bound fraction to the starting sample (step 3).
7. Wash resin with 6mL of pH 7.2 Coupling Buffer. Replace bottom cap when buffer drains to top of resin bed.
8. In a fume hood, add 2mL of pH 7.2 Coupling Buffer and 40µL of Cyanoborohydride Solution to the reaction slurry (results in ~50mM $NaCNBH_3$ when mixed with resin).
9. Replace top cap and mix column for 4 hours at room temperature or overnight at 4°C.
10. In a fume hood, carefully remove the top cap. Some gas pressure may have formed during the reaction.
11. Remove the bottom cap, place the column in a new collection tube and allow it to drain.

B. Block Remaining Active Sites

1. Wash resin with 4mL of Quenching Buffer, and then replace the bottom cap.
2. In a fume hood, add 2mL of Quenching Buffer and 40µL of Cyanoborohydride Solution to the column (results in ~50mM $NaCNBH_3$ when mixed with resin). Replace the top cap and mix gently for 30 minutes by end-over-end rocking.
3. In a fume hood, carefully remove the top cap. Some gas pressure may have formed during the reaction.
4. Remove bottom cap, place the column in a new collection tube and allow it to drain.

C. Wash Column

1. Wash column with at least 10mL (5 resin-bed volumes) of Wash Solution.

   Note: Monitor the final washes for the presence of protein. Although the washes should remove all non-coupled protein, proteins coupled at high concentrations or at pH 10 may require extensive washing for complete removal.
2. Wash the resin with 6mL of degassed buffer containing 0.05% sodium azide or other preservative. Replace bottom cap while there is still at least 1mL of buffer covering the top of resin bed. Store column upright at 4°C.

   Note: If desired, position a porous disc just above the top of the resin bed. The disc prevents resuspension of packed resin bed when adding solution to the column and it protects the column from drying by automatically stopping column flow when solution drains down to the top of the disc.

Procedure for Coupling Protein Using the pH 7.2 Coupling Buffer

1. Equilibrate upright column containing desired amount of AminoLink Plus Resin to room temperature and allow the resin to settle. Open column and drain storage solution into a collection tube. Throughout entire procedure, do not allow the resin bed to become dry. Place bottom cap on the column when the buffer drains down to the top of the resin bed.
2. Equilibrate column by adding 6mL (3 resin-bed volumes) of pH 7.2 Coupling Buffer and allowing the contents to drain.
3. Replace the bottom cap and add 2-4mL of the protein solution (dissolved in pH 7.2 Coupling Buffer) to the column. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.
4. In a fume hood, add 40µL of Cyanoborohydride Solution to the reaction slurry (results in ~50mM $NaCNBH_3$).

5. Place the top cap on the column and mix the reaction by end-over-end rocking for 6 hours at room temperature or overnight at 4°C. For proteins that are sensitive to agitation, mix for 2 hours and then allow column to remain stationary for an additional 4 hours.
6. In a fume hood, carefully remove the top cap. Some gas pressure may have formed during the reaction.
7. Remove the bottom cap and drain the contents of the column into a new collection tube.
8. Save the flow-through and determine the coupling efficiency while continuing with column blocking steps. Determine coupling efficiency by comparing the protein concentrations of the non-bound fraction to the starting sample (step 3).
9. Wash resin with 4mL of pH 7.2 Coupling Buffer. Replace the bottom cap.
10. Proceed with Sections B and C of the **Procedure for Coupling Protein to Column Using the pH 10 Coupling Buffer** (Block Remaining Active Sites and Wash Column).

General Protocol for Affinity Purification of Protein

Note: This protocol uses a gravity-flow column with a resin-bed volume of 2mL. 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. For a centrifuge column procedure, see instructions for Product No. 44894.

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: 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: Degas all buffers to avoid introducing air bubbles into the column. 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 allow excess storage solution to drain from column.
3. Equilibrate column by adding 6mL of Binding/Wash Buffer and allowing it to drain from column.
4. Add Sample to column and allow it to flow into the resin bed. For samples < 2mL, extend binding time by replacing the bottom cap to stop flow for a time (e.g., 1 hour) after the sample has entered resin bed. For samples > 2mL, extend binding time by capping to stop column flow after each 2mL volume of sample has passed into/through the resin bed.
5. Remove top cap and bottom caps from column, place column in new collection tube, and wash the column with 12mL of Binding/Wash Buffer.
6. 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.
7. Monitor elution by absorbance at 280nm. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.

C. Column Regeneration and Storage

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

1. Wash column with 16mL of Binding/Wash Buffer to remove any residual protein and reactivate the resin.
2. Equilibrate column with 8mL 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	Cause	Solution
Low coupling efficiency	Primary amines not completely removed from sample before coupling	Ensure primary amines have been completely removed by extensive dialysis or desalting
Protein to be immobilized is not soluble in Coupling Buffer	Molecule was hydrophobic	Dissolve molecule in Coupling Buffer containing up to 4M guanidine•HCl or 20% DMSO (see Additional Information section)
Column flows slowly	Air bubbles in column were restricting flow (air bubbles may not be visible)	Remove air bubbles by stirring or centrifugation (see Additional Information section)
Affinity column loses binding capacity over time	Immobilized sample was damaged by time, temperature or elution conditions	Prepare a new affinity column and alter the procedure responsible for damaging the column
	Nonspecifically bound material had reduced capacity	Wash column with high salt (~1M NaCl) to remove nonspecifically bound material
		Remove precipitate from sample before affinity purification by centrifugation of 0.45µm filter

Related Thermo Scientific Products

89896 **Pierce® Centrifuge Columns, 2mL,** 25 units

89897 **Pierce Centrifuge Columns, 5mL,** 25 units

89898 **Pierce Centrifuge Columns, 10mL,** 25 units

44892 **AminoLink Reductant,** Sodium cyanoborohydride ($NaCNBH_3$), 2 × 1g

28372 **BupH™ Phosphate Buffered Saline (PBS),** each dry-blend pack makes 500 ml with water, 40 packs

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

Additional Information Available on Our Website

- Centrifuge Protocol: See instructions for the related AminoLink Plus Immobilization Kit (Product No. 44894)
- 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 buffers for use in affinity and gel filtration columns

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.

Hornsey, V.S., *et al.* (1986). Reductive amination for solid-phase coupling of protein. A practical alternative to cyanogen bromide. *J Immunol Methods* **93(1)**:83-8.

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