INSTRUCTIONS

AminoLink® Coupling Gel

PIERCE Biotechnology
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20381 20382 0402w

Number	Description
20381	**AminoLink® Coupling Gel**, 10 ml resin, 20 ml slurry
20382	**AminoLink® Coupling Gel**, 50 ml resin, 100 ml slurry
	Support: 4% cross-linked beaded agarose
	Supplied as a 50% slurry in 0.02% sodium azide.

Storage: Upon arrival store at 4°C. Product is shipped at ambient temperature.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

Introduction

AminoLink® Coupling Gel allows the simple and efficient immobilization of a wide variety of proteins to a solid support. When the AminoLink® Coupling Gel reacts with a protein, the result is a stable and reusable affinity column that provides a valuable tool for the purification of an antibody, antigen or other biomolecule. AminoLink® Coupling Gel covalently links antibodies, antigens or other proteins over a wide range of temperature, pH and buffer conditions. Average coupling efficiencies with AminoLink® Coupling Gel are approximately 80% from pH 4-10. Furthermore, AminoLink® Coupling Gel efficiently couples proteins regardless of their molecular weight or pI.

The AminoLink® Coupling Gel support is 4% cross-linked beaded agarose that has been activated to form aldehyde functional groups. The aldehydes react spontaneously with primary amines found in lysine residues and at the amino terminus of a peptide chain. Reductive amination of the resulting Schiff base then forms a stable, secondary amine linkage with minimal leakage of the ligand.

Procedure for Immobilizing a Protein

Note: the procedure presented here is for batch method (small-scale) coupling of ligand to the gel. A larger scale procedure for coupling and use in column format is presented in the instructions for Product No. 20501 (see Related Pierce Products).

A. Additional Materials Required

- Coupling Buffer: 0.1 M sodium phosphate buffer, 0.05% sodium azide, pH 7.0-7.5
- Cyanoborohydride Solution ($NaCNBH_3$, see Related Pierce Products): 1 M $NaCNBH_3$ in 0.01 N NaOH

  Note: This should be prepared in a fume hood because $NaCNBH_3$ is toxic
- Quenching Buffer: 1 M Tris•HCl, pH 7.4
- Washing Buffer: 1 M sodium chloride (NaCl)
- Storage Buffer: Phosphate Buffered Saline (PBS, see Related Pierce Products) or other suitable storage buffer containing 0.05% sodium azide

B. Sample Preparation

Dissolve the protein to be immobilized in Coupling Buffer. Appropriate coupling reaction loadings are 1-20 mg protein per ml of AminoLink® Coupling Gel or 1-2 mg peptide per ml of AminoLink® Coupling Gel. Coupling reactions composed of equal volumes of sample and gel are commonly used; however, sample volumes up to 10 times greater than the gel volume are acceptable.

Note: For proteins already in solution, make a 1:3 dilution of the sample in Coupling Buffer or dialyze into the Coupling Buffer. However, if the buffer contains primary amines (e.g., Tris or glycine), these compounds must be thoroughly removed before coupling because they will quench the coupling reaction.

C. Gel Preparation

1. Resuspend the AminoLink® Coupling Gel by inverting the bottle several times.
2. Transfer a volume of the gel slurry equal to twice the desired gel volume into a separate tube. For example, for 500 µl of gel, transfer 1 ml of slurry.
3. Wash the gel 3 times with Coupling Buffer, pelleting the gel and removing the supernatant after each wash.

   Note: For wash steps, use approximately 10 times the gel volume.

D. Coupling Protein to Gel

1. Add the sample solution prepared in Step B to the AminoLink® Coupling Gel.
2. In a fume hood, add 25 µl of Cyanoborohydride Solution per ml of the reaction volume (sample volume + gel volume).
3. Cap the tube and mix for 6 hours at room temperature.

   Note: Incubations may be carried out overnight at 4°C. For proteins that are sensitive to agitation, mix for only 2 hours, then let the tube sit without rocking for an additional 4 hours.
4. Carefully remove the top cap (some gas pressure may have built up during the reaction) and decant supernatant.

E. Blocking Remaining Active Sites

1. Wash the gel once with Quenching Buffer (1 M Tris).
2. Add a volume of Quenching Buffer equal to the gel volume.
3. In a fume hood, add 50 µl of Cyanoborohydride Solution per ml of gel.
4. Cap the tube and mix for 30 minutes at room temperature.

F. Washing and Storage

1. Pellet the gel and decant the supernatant.
2. Wash the gel several times with Wash Buffer (1 M NaCl).

   Note: Monitor the final washes for the presence of protein. Proteins coupled at high concentrations may require more washing for complete removal of uncoupled protein.
3. Wash the gel with degassed water or buffer containing 0.05% sodium azide.
4. Replace the cap and store at 4°C.

The protein is now covalently coupled to the AminoLink® Coupling Gel and can be used for affinity purification.

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/peptide to be immobilized is not soluble in coupling buffer	Molecule is hydrophobic	Dissolve molecule in coupling buffer containing up to 4 M guanidine•HCl or 20% DMSO
Affinity column loses binding capacity over time	Immobilized sample has been damaged through time, temperature or elution conditions	Prepare a new affinity column and alter the procedure responsible for damage to the column
	Column has become fouled with non-specifically bound material	Wash column with high salt (~1 M NaCl) to remove nonspecifically bound material
		Prepare a new affinity column and remove foulants from sample before affinity purification

Related Pierce Products

Number	Description
44892	**AminoLink® Reductant**, (sodium cyanoborohydride), 2 gm
20501	**AminoLink® Plus Coupling Gel**, aldehyde-activated gel for coupling ligands through primary amines; when used in column format, gel has faster flow rate than regular AminoLink® Coupling Gel.
28372	**BupH™ Phosphate Buffered Saline Packs**, 40 packs, each pack yields 0.1 M phosphate, 0.15 M NaCl, pH 7.2 solution when reconstituted with 500 ml water

Additional Information

Please visit the Pierce web site for additional information on this product including the following items:

- Tech Tip protocol: Optimize Elution Conditions for Immunoaffinity Chromatography
- Tech Tip protocol: Remove Air Bubbles from Columns
- Tech Tip protocol: Degas Solutions for use in Affinity Columns

References

An excellent overview is found in "Immobilized Affinity Ligand Techniques" by Hermanson, *et al.*, Product No. 22230.

1. Lasch, J. and Koelsch, R. (1978). Enzyme leakage and multipoint attachment of agarose-bound enzyme preparations. *Eur. J. Biochem*. **82**, 181-186.
2. Domen, P., Nevens, J., Mallia, K., Hermanson, G. and Klenk, D. (1990). Site directed immobilization of proteins. *J. Chromatogr.* **510**, 293-302.
3. Hermanson, G.T., Mallia, K.A., Smith, P.K. (1992). *Immobilized Affinity Ligand Techniques*. Academic Press, Inc.: San Diego, CA.