INSTRUCTIONS

Thermo SCIENTIFIC

AminoLink® Plus Immobilization Kit

44894 20394

0491.10

Number	Description
44894	**AminoLink Plus Immobilization Kit**, sufficient reagents to prepare five affinity columns

Kit Contents:

AminoLink Plus Column, 5 × 2mL, 4% beaded agarose supplied as 50% slurry in 0.02% sodium azide (NaN_3)

BupH™ Phosphate Buffered Saline Pack, 1 pack, yields 0.1M phosphate, 0.15M sodium chloride, pH 7.2, when reconstituted with 500mL of water (pH 7.2 Coupling Buffer)

BupH Citrate-Carbonate Buffer, 1 pack, yields 0.1M sodium citrate, 0.05M sodium carbonate, pH 10.0, when reconstituted with 500mL of water (pH 10 Coupling Buffer)

Quenching Buffer, 50mL, 1M Tris•HCl, 0.05% NaN_3, pH 7.4

Wash Solution, 240mL, 1M NaCl, 0.05% NaN_3

Sodium Cyanoborohydride Solution (5M), 0.5mL, $NaCNBH_3$ (MW 62.84) dissolved in 1M NaOH

Column Accessories, porous discs (6), white tips (10), separator and column extender

Number	Description
20394	**AminoLink Plus Immobilization Trial Kit**, sufficient reagents for preparing one affinity column

Kit Contents:

AminoLink Plus Column, 2mL, 4% beaded agarose supplied as 50% slurry in 0.02% sodium azide (NaN_3)

BupH Phosphate Buffered Saline Pack, 1 pack, yields 0.1M phosphate, 0.15M NaCl; pH 7.2, when reconstituted with 500mL of water

BupH Citrate-Carbonate Buffer, 1 pack, yields 0.1M sodium citrate, 0.05M sodium carbonate; pH 10.0, when reconstituted with 500mL of water

Quenching Buffer, 15mL, 1M Tris•HCl, 0.05% NaN_3, pH 7.4

Wash Solution, 50mL, 1M NaCl, 0.05% NaN_3

Sodium Cyanoborohydride Solution (5M), 0.5mL, $NaCNBH_3$ (MW 62.84) dissolved in 1M NaOH

Column Accessories, porous discs (2), white tips (2), resin separator and column extender

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

Introduction

The Thermo Scientific AminoLink Plus Immobilization Kit contains all the necessary components for covalent attachment of primary amine-containing proteins and other ligands to a beaded agarose support. The result of protein immobilization is a stable and reusable column for affinity purification of antibodies, antigens and other biomolecules. The kit features spin columns and procedures that provide excellent performance and greater speed and convenience than strictly gravity-flow methods.

The AminoLink Plus Resin contains aldehyde groups that react specifically with primary amines (-NH_2), which occur on the surface of proteins in the side chains of lysine (K) residues and the N-terminus of each polypeptide. After the spontaneous formation of semi-stable Schiff base bonds, reduction with sodium cyanoborohydride results in stable secondary amine bonds (Figure 1). Antibodies, antigens and other proteins having different molecular weights and isoelectric points couple to AminoLink Resin with an average coupling efficiency greater than 80%.

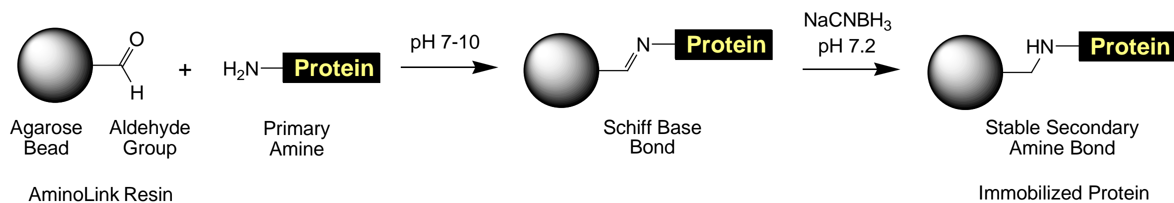

Figure 1. General structure and reaction scheme for AminoLink Plus Resin.

Important Product Information

- Equilibrate kit components to room temperature before processing.
- To quantify the protein and estimate coupling efficiency use a spectrophotometer or other method of choice.
- Hydrophobic proteins may require additional wash steps or addition of detergent during coupling to minimize nonspecific binding.
- Particulate material in samples can impede column flow. Remove particulate by centrifugation (10,000 × *g*) or filtration (0.45µm) before applying the sample to the column.
- Perform steps involving the handling of sodium cyanoborohydride solutions in a fume hood and wear protective gloves.
- Although the entire coupling reaction is effective over a wide pH range, 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.

Material Preparation

Coupling Buffers: Prepare pH 7.2 and pH 10 buffers by dissolving BupH Pack contents in 500mL of ultrapure water. For storage of excess buffer, add a suitable preservative such as 0.05% sodium azide and store at 4°C.

Protein Sample: 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.

Storage Buffer: Prepare phosphate-buffered saline (PBS; same as pH 7.2 coupling buffer) containing 0.05% sodium azide (NaN_3). Alternative buffers and preservatives that are suitable for storage of the immobilized protein may be used.

Procedure for Coupling Protein Using the pH 10 Coupling Buffer

A. Protein Immobilization

Note: Perform all AminoLink Plus Column centrifugations at 1000 × *g* for 1 minute using a 15mL collection tube. Do not allow the AminoLink Plus Resin to become dry at any time.

1. Suspend the AminoLink Plus Resin by end-over-end mixing. To avoid drawing air into the column, sequentially remove the top cap and then the bottom tab. Centrifuge the column to remove the storage buffer.
2. Add 2mL of pH 10 Coupling Buffer and centrifuge. Repeat with an additional 2mL of pH 10 Coupling Buffer.
3. Replace the bottom cap. Add 2-3mL of the Protein Sample that was dissolved in pH 10 Coupling Buffer to the AminoLink Plus Column. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.
4. Replace the top cap and mix column by rocking or end-over-end mixing at room temperature for 4 hours or overnight.

   Note: For proteins that are sensitive to long-term agitation (e.g., precipitate), rock 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 column caps, place column into a new tube and centrifuge to collect non-bound protein.

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 the column with 2 mL of pH 7.2 Coupling Buffer (PBS) and centrifuge. Repeat this step once.
8. Replace the bottom cap. In a fume hood, add 2mL of pH 7.2 Coupling Buffer to 40µL of Sodium Cyanoborohydride Solution, and add the resulting solution to the column (results in 50mM $NaCNBH_3$ when mixing with resin).
9. Replace the top cap and mix column for 4 hours at room temperature or overnight at 4°C.

B. Block Remaining Active Sites

1. Carefully remove the top cap. Some gas pressure may have formed during the reaction.
2. Remove bottom column cap, place column into a new tube and centrifuge to remove Coupling Buffer.
3. Wash the column with 2mL of Quenching Buffer and centrifuge. Repeat this step once.
4. Replace bottom cap. In a fume hood, add 2mL of Quenching Buffer to 40µL of Sodium Cyanoborohydride Solution, and add the resulting solution to the column (results in 50mM $NaCNBH_3$ when mixed with resin).
5. Replace the top cap and mix gently for 30 minutes by end-over-end rocking.

C. Wash Column

1. Carefully remove the top cap. Some gas pressure may have formed during the reaction.
2. Remove bottom column cap, place column into a new tube and centrifuge to remove Quenching Buffer.
3. Wash away reactants and noncoupled protein with 2mL of Wash Solution and centrifuge. Repeat this step four times.

   Note: Monitor wash for the presence of protein. Usually washing with 10mL will to remove all non-coupled protein, but proteins coupled at high concentrations or at pH 10 may require more extensive washing for complete removal.
4. Equilibrate column for storage: add 2mL of Storage Buffer and centrifuge. Repeat this step two times.
5. Replace the bottom cap and add 2mL of Storage Buffer to the top of the resin bed.
6. Proceed with step B.3 of the General Protocol for Affinity Purification or replace the top cap and store upright at 4°C.

Procedure for Coupling Protein Using the pH 7.2 Coupling Buffer

Note: Perform all AminoLink Plus Column centrifugations at 1000 × *g* for 1 minute using a 15mL collection tube. Do not allow the AminoLink Plus Resin to become dry at any time.

1. Suspend the AminoLink Plus Resin by end-over-end mixing. To avoid drawing air into the column, sequentially remove the top cap and then the bottom tab. Centrifuge the column to remove the storage buffer.
2. Add 2mL of pH 7.2 Coupling Buffer and centrifuge. Repeat with an additional 2mL of pH 7.2 Coupling Buffer.
3. Replace the bottom cap. Add 2-3mL of the Protein Sample that was dissolved in pH 7.2 Coupling Buffer to the AminoLink Plus Column. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.
4. In a fume hood add 40µL of Sodium Cyanoborohydride Solution to the reaction slurry (results in ~50mM $NaCNBH_3$).
5. Replace the top cap and mix column by rocking or end-over-end mixing at room temperature for 4 hours or overnight.

   Note: For proteins that are sensitive to long-term agitation (e.g., precipitate), rock 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.
6. Remove top and bottom column caps, place column into a new tube and centrifuge to collect non-bound protein.
7. 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).
8. 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

The following protocol details a spin-purification method; if desired, the traditional gravity-flow method can be used instead. The amount of protein sample that can be processed and the binding conditions required depend on the specific affinity interaction used and must be optimized for the particular experiment.

Note: For gravity-flow methods, a porous disc placed just above the resin bed automatically stops column flow when the solution has drained down to the top of the resin bed, preventing the column from drying. The disc also prevents resuspension of the packed bed when adding solution to the column. To insert the disc, use the open tube end of a resin separator to slide it to within 1 mm of the resin bed.

Materials Required

- Binding/Wash Buffer: Use phosphate-buffered saline (PBS; Product No. 28372), Tris-buffered saline (TBS; Product No. 28379) or other buffer that is conducive to forming the intended affinity interaction. Degas buffers to avoid introducing bubbles into the resin bed that may impede flow.
- Sample: Dissolve or exchange sample into Binding/Wash Buffer
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl at pH 2.5-3.0
- Neutralization Buffer (optional): Prepare 1mL of 1M sodium phosphate or 1M Tris•HCl at pH 8.5-9.0

Method

Note: Perform all centrifugations at 1000 × *g* for 1 minute using a 15mL collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove top and bottom column caps. Centrifuge column to remove storage solution. Equilibrate column with 6mL of Binding/Wash Buffer.
3. Add sample (≤ 2mL) in appropriate binding buffer to the column. Allow sample to enter the resin bed and replace bottom cap. Add 0.2mL of Binding/Wash Buffer. Replace top cap and incubate column at room temperature while rocking to allow binding to occur (e.g., 15-60 minutes). For samples > 2mL, add volumes in succession or process sample by batch method.
4. Remove top and bottom caps and centrifuge column. Without changing collection tubes, add 1mL of Binding/Wash Buffer and centrifuge again. Save the entire flow-through to evaluate binding efficiency and capacity.
5. To wash the resin, add 2mL of Binding/Wash Buffer and centrifuge. Repeat this step 2-4 times.
6. Elute the protein with 2mL of Elution Buffer collecting into a centrifuge tube containing 100µL of Neutralization Buffer and centrifuge. Save eluted, neutralized sample and repeat this step 2-3 times.
7. Use the protein directly for SDS-PAGE or analyze by protein assay. If required for the specific downstream assay or storage, perform a buffer exchange by dialysis or gel filtration.

   Note: Equilibrate the column soon after use to prevent damage to the immobilized protein by the low pH Elution Buffer. Typically, an affinity column can be reused ~10 times, depending on the stability of the immobilized molecule.
8. To equilibrate the column, pass 4mL of Binding/Wash Buffer through the column.
9. Cap the bottom and add 4mL of Binding/Wash Buffer that contains a final concentration of 0.05% sodium azide for long-term storage. Cap the top and store column upright at 4°C. Do not freeze the resin.

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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)
Affinity column has reduced binding capacity after several uses	Immobilized protein was damaged by time, temperature or elution conditions	Prepare a new affinity column
	Binding sites and resin pores were blocked with particulate or nonspecifically bound material	Remove precipitate from sample before affinity purification by centrifugation or 0.45µm filter
		Use nonionic detergent, high salt concentration, or other additives to reduce nonspecific binding or wash with greater stringency before elution

Additional Information Available on Our Website

- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip #43: Protein stability and storage
- Tech Tip #12: Prepare molecules with poor solubility for immobilization on affinity supports

Related Thermo Scientific Products

20501 **AminoLink Plus Coupling Resin,** 10mL

21004 **IgG Elution Buffer**, 1L

21027 **Gentle Ag/Ab Elution Buffer,** 500mL

28372 **BupH Phosphate Buffered Saline Packs**, 40 packs

78501 **M-PER® Mammalian Protein Extraction Reagent,** 250mL

20475 **AminoLink Plus Micro Immobilization Kit, 0.1mL**

General References

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.

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.

This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”).

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