

# AminoLink<sup>®</sup> Immobilization Kit

44890

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**Number**

44890

**Description**

**AminoLink Immobilization Kit**, sufficient reagents to prepare five affinity columns

**Kit Contents:**

**AminoLink Columns**, 5 × 2mL, crosslinked 4% beaded agarose supplied as 50% slurry in 0.02% sodium azide (NaN<sub>3</sub>)

**AminoLink Coupling Buffer**, 250mL, 0.1M sodium phosphate, 0.05% NaN<sub>3</sub>, pH 7.0

**Quenching Buffer**, 50mL, 1M Tris•HCl, 0.05% NaN<sub>3</sub>, pH 7.4

**Wash Solution**, 240mL, 1M NaCl, 0.05% NaN<sub>3</sub>

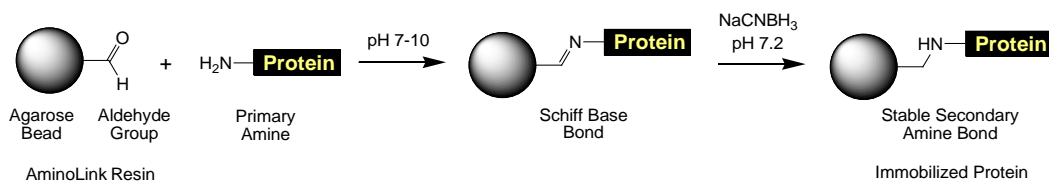
**Sodium Cyanoborohydride Solution (5M)**, 0.5mL, NaCNBH<sub>3</sub> (MW 62.84) dissolved in 1M NaOH

**Column Accessories**, porous discs (6), white tips (5), resin separator and column extender

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

**Introduction**

The Thermo Scientific AminoLink Immobilization Kit 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 (Figure 1). Coupling efficiency by this reductive amination mechanism is typically greater than 80%, regardless of the ligand's molecular weight or pI (Table 1). Once the ligand is immobilized, the prepared resin can be used for multiple rounds of affinity purification in simple gravity-flow, batch or small spin column procedures.



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

**Table 1.** Coupling efficiency of various proteins using AminoLink Coupling Resin. For each experiment, 20mg of protein was reacted with 2mL AminoLink Coupling Resin using the standard protocol.

<b>Protein</b>	<b>Protein pI</b>	<b>Molecular Weight</b>	<b>Coupling Efficiency (%)</b>
Ovalbumin	4.7	45,000	52
A1-Antitrypsin	4.0	--	66
Fetuin	3.3	--	74
IgG (bovine)	--	150,000	79
Cytochrome C	9.0-9.4	11,700	79
Myoglobin	6.8-7.8	16,900	84
α-Lactalbumin	5.2	35,000	84
Transferrin	5.9	85,000	88
Hemoglobin	6.8	64,500	91
Ribonuclease A	9.5	13,700	92
Lysozyme	11.0	14,000	96

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## Procedure for Immobilizing a Protein to AminoLink Column

### A. Protein Sample Preparation

Dissolve 1-20mg protein or 1-2mg peptide to be immobilized in 2-3mL of Coupling Buffer. 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.

### B. Protein Immobilization Reaction

1. Equilibrate upright AminoLink Column to room temperature and allow the resin to settle. Remove top cap, twist off bottom tab, and drain storage solution into a collection tube. Throughout entire procedure, do not allow the resin bed to become dry. Place bottom cap (white tips supplied) on column when the buffer drains down to the top of the resin bed.
2. Equilibrate column by adding 6mL (3 resin-bed volumes) of Coupling Buffer and allowing the contents to drain.
3. Replace bottom cap (supplied white tip) and add 2-4mL of the protein solution (dissolved in Coupling Buffer) to the column. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.
4. In a fume hood, add 10 $\mu$ L of Sodium Cyanoborohydride Solution per milliliter of total volume of reaction slurry (resin + sample). Results in ~50mM NaCNBH<sub>3</sub>.
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 Coupling Buffer.

### C. 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 40 $\mu$ L of Sodium Cyanoborohydride Solution to the column (results in ~50mM NaCNBH<sub>3</sub> 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.

### D. 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 high pH 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 suspension of packed resin bed when adding solution to the column and protects the column from drying by automatically stopping column flow when solution drains down to the top of the disc.

## General Protocol for Affinity Purification of Protein

**Note:** This protocol uses of 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.

### 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. Affinity Purification 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 6 mL 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 < 2 mL, 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 is 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 are 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 has 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

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## Related Thermo Scientific Products

89897	Pierce® Centrifuge Columns, 5mL (resin bed capacity), 25/pkg
44892	AminoLink Reductant, Sodium cyanoborohydride (NaCNBH <sub>3</sub> ), 2 × 1g
28372	BupH™ Phosphate Buffered Saline (PBS), 40 packs
21004	IgG Elution Buffer, 1L

## Additional Information

Visit the website for additional information relating to this product including the following items:

- 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

## References

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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.

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