Troubleshooting affinity chromatography

Problem: Tagged protein appears in washes, does not bind to affinity resin

Possible cause	Remedy
Antibody did not couple to support resin	Try another method to couple the antibody to the resin.
Antibody binding site altered or blocked during antibody-resin coupling procedure; cannot bind tagged protein	Use a different chemical technique to couple the antibody to the resin. For example, couple the antibody through its carbohydrate groups (hydrazide coupling) rather than through its amino groups.
One or more of these sample loading conditions may not be optimal for binding tagged protein to affinity column: • pH • Flow rate • Temperature • Salt or ion concentration	Do trial experiments to optimize loading parameters. Follow these general guidelines: • Load at neutral pH. • Load sample slowly or use batch loading. • Load at room temperature. • Salt and ion concentration in Binding Buffer should be kept low.
Proteases are degrading tagged protein	Add protease inhibitors (BM) to sample loading/wash buffer. *Note: See Section 4B of this manual for more information on protease inhibitors.*
Antibody on affinity column was inactivated during prior use	Always wash column with Binding Buffer right after each use. Always store column at 4°C in Binding Buffer + Azide. Prepare a new column.
Channels have formed in column bed so loaded sample runs through column without interacting with antibody	Re-pack column.

Problem: Tagged protein remains bound to column, does not elute in Elution Buffer.

Possible cause	Remedy
Elution Buffer is not optimal	Try alternative elution buffers, such as described in Procedure II. Try a combination of the elution buffers described in Procedure II. Try elution at a higher temperature.

Problem: Extra proteins in eluant with tagged protein

Possible cause	Remedy
Nonspecific proteins binding to affinity resin	Before applying sample the first time, block the resin with a protein that will not later interfere with the chromatography, for instance, bovine serum albumin.
	After sample application, wash column more thoroughly before eluting tagged protein.
	Wash columns with a more stringent second Wash Buffer (for example, with higher salt).
	Apply sample in a Binding Buffer containing enough salt to minimize nonspecific binding (but not the binding of the tagged protein).

Problem: Tagged protein degraded

Possible cause	Remedy
Proteases in sample	Include protease inhibitors throughout procedures. Work at low temperature, such as 4°C, to minimize degradation.
Tagged protein unstable	Work at low temperature, such as 4°C, to minimize degradation.

Problem: Bubbles in affinity column

Possible cause	Remedy
Column poured and stored at one temperature, but used at another	Always allow column to equilibrate to procedure temperature, then degas under vacuum and remix resin to remove bubbles before using. OR Pour column at the same temperature that it will be used. OR Pour column at a warmer temperature, then let it equilibrate to a cooler temperature. Use the column at the cooler temperature.

Problem: Tagged protein elutes as a diffuse band

Possible cause	Remedy
Elution Buffer does not immediately release protein from resin	Increase concentration of release agent (peptide, salt, etc.) in Elution Buffer.
Elution causes band diffusion	Use reverse (upward) flow to elute the column. *(Note: requires mechanical pump to create upward flow.)*

Suggested reading to learn more about procedure

There are numerous affinity purification protocols described in the literature. For a detailed description of these techniques and discussion of factors affecting the results, see Hermanson, Mallia and Smith (1992). Other helpful references include Cuatrecases, Wilchek and Anfinsen (1968); Dean, Johnson and Middle (1985); Harlow and Lane (1988); and Wilchek, Miron and Kohn (1984).

References

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