

Section 4C

Purification by Affinity Chromatography

Overview of technique

In immunoaffinity chromatography, an antibody against the epitope-tagged portion of the fusion protein is covalently attached to a support resin. Affinity chromatography (Figure 4C.1) allows purification of large amounts of fusion protein from cell lysates and supernatants (Templeton, 1992; Field *et al.*, 1988).

Affinity chromatography offers the following advantages over other chromatography methods:

- ▶ Saves time by providing a highly purified epitope-tagged protein in a single step
- ▶ Preserves protein activity by using gentle elution conditions
- ▶ Produces highly purified tagged protein

The advantages of the immunoaffinity method often offset the initial high cost and labor involved in making your own immunoaffinity resin.

Note: For detailed, step-by-step affinity chromatography procedures, see Hermanson, Mallia and Smith (1992).

Getting started: Procedures for affinity chromatography

We describe below general guidelines for making and using your own immunoaffinity resin, that is, for attaching any tag-specific antibody to a support resin and purifying the corresponding epitope-tagged protein (Figure 4C.2).

Caution: The guidelines given in these procedures are only a starting point for developing an affinity purification system and have not been optimized for a particular epitope tag or tag-specific antibody. Optimize these procedures experimentally to account for the variables introduced by different tagged proteins, antibodies, and chromatography methods.

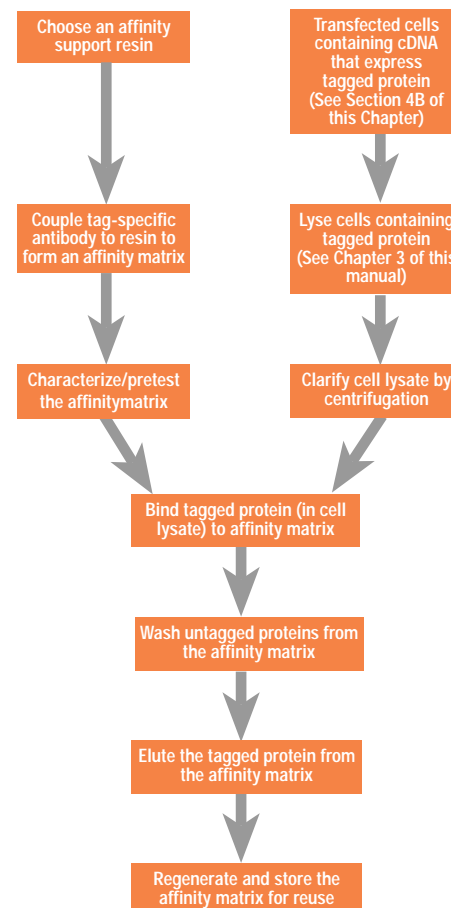


Figure 4C.2: Flow diagram for developing and using an affinity purification system for an epitope-tagged protein. Details are given in the text.

Note: The products printed in *colored type* are available from Boehringer Mannheim. For detailed ordering information on these and related products, see the Boehringer Mannheim Product Ordering Guide, Section 5B of this manual.

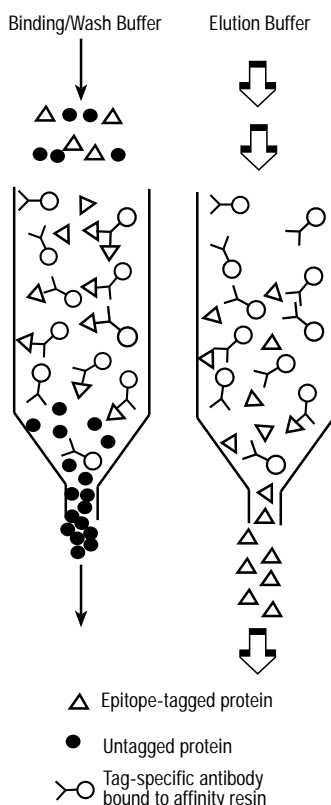


Figure 4C.1: How affinity chromatography works. A mixture of proteins in a Binding/Wash Buffer is passed over affinity resin beads that contain covalently bound tag-specific antibody. Untagged proteins (●) pass through the column, while tagged proteins (△) are captured by the tag-specific antibody bound to the beads. An Elution Buffer releases the tagged protein from the beads.