

Read this chapter to learn more about these topics:

Topic	See page
Overview of Immunoaffinity Purification Methods	4.3
Critical Factors for Successful Purification of Tagged Proteins	4.4
Purification by Affinity Chromatography	4.6
Purification by Immunoprecipitation	4.15

# Immunoaffinity Purification of Tagged Proteins

# 4

## Section 4A

# Overview of Immunoaffinity Purification Methods

Immunoaffinity purification, or purification by antibody-antigen interaction, is a powerful tool for quickly and gently isolating an epitope-tagged protein from a crude cell extract.

Epitope-tagged proteins can be isolated by two distinct immunoaffinity purification techniques:

**Affinity Chromatography:** The antibody (in this case, a tag-specific antibody) used to pull the tagged protein out of a complex mixture is covalently attached to a solid support resin. Then, a cell extract containing the tagged protein is passed over the attached antibody-affinity resin. While the tagged protein binds to the antibody, untagged proteins and other cell components simply pass through the column. Once the unbound proteins are washed off the column, a specific eluant (such as a buffer containing an epitope peptide recognized by the attached antibody) can release the bound tagged protein from the column. (See Section 4C, “Purification by Affinity Chromatography” in this chapter.)

**Immunoprecipitation:** The purifying antibody (tag-specific antibody) binds to the target (tagged) protein in solution. Then the antibody-antigen complex is pulled out of the complex mixture with an insoluble matrix which has an affinity for the tag-specific antibody (for example, protein A-agarose, protein G-agarose, *Staphylococcus aureus* cells, or affinity resin with covalently attached secondary antibody). Proteins which do not bind to the tag-specific antibody or the insoluble matrix are left behind in solution. Then, a specific reagent (for instance, a competing epitope tag peptide or a detergent) releases the bound tagged protein from the insoluble matrix-antibody-antigen complex. (See Section 4D, “Purification by Immunoprecipitation” in this chapter.)

### Choosing an immunoaffinity purification technique

Table 4A.1 summarizes the characteristics of the two types of techniques.

	Affinity chromatography	Immunoprecipitation
<b>Amount of protein that can be purified</b>	Large amounts (milligram) can easily be purified from a single sample	Only small amounts (microgram or less) can be readily purified from each sample
<b>Number of samples processed in a single experiment</b>	One or a few	Many samples possible
<b>Time required</b>	Days, including synthesis of matrix containing attached antibody	Hours, using off-the-shelf reagents
<b>Expense</b>	Relatively high; requires large (milligram) amounts of tag-specific antibody	Relatively low, requires small (microgram) amounts of tag-specific antibody

Table 4A.1: Guidelines for choosing an immunoaffinity purification technique

Usually, the choice of one technique over the other is dictated by two things: the number of samples that need to be purified simultaneously, and the amount of protein in each sample.

If you need to purify large amounts of tag-specific protein from a single sample, choose affinity chromatography.

However, if you need to analyze tagged protein from a series of small, related samples, choose immunoprecipitation.

**Note:** Obviously, these generalizations have exceptions. Affinity chromatography can be adapted to process multiple samples (as in Procedure VI, Section 4C of this chapter). Immunoprecipitation techniques can be scaled up.