

Section 4B

Critical Factors for Successful Analysis and Purification of Tagged Proteins

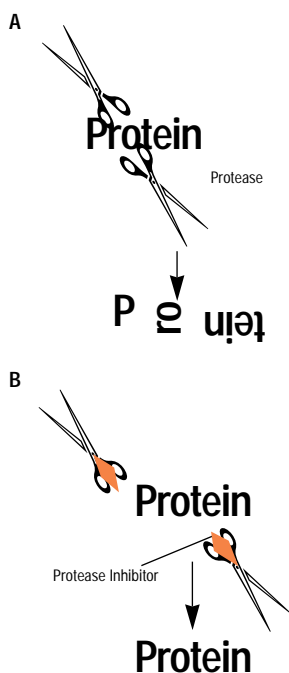


Figure 4B.1: Proteases and protease inhibitors. Proteases (panel A) can cleave a target protein into several pieces, destroying its native structure. Protease inhibitors (panel B) block the activity of the proteases and leave the target protein intact.

Cell lysis, solubilization of proteins, and prevention of proteolytic degradation of proteins are crucial steps in immunoaffinity purification.

Preventing proteolysis during sample purification

Disruption of almost any kind of cell releases proteases. These proteases, if not inactivated, may hydrolyze the proteins they contact (Figure 4B.1). Multidomain proteins (such as epitope-tagged or fusion proteins) may be especially vulnerable to proteolysis.

Thus, for any procedure that requires cell lysis, you must take steps to prevent proteolysis and degradation of the tagged protein. When proteases are involved, the safest advice is:

- ▶ Always assume proteases are present and active, even in the presence of harsh, denaturing detergents such as sodium dodecyl sulfate (SDS).
- ▶ Add protease inhibitors (Figure 4B.1) to lysis buffer just before the buffer contacts the protein; do not add inhibitors to a stock buffer days or weeks before it is used.
- ▶ Wherever possible, perform procedures at low temperatures, on ice, or at 4°C, where protease activity is much lower than at elevated temperatures.

What proteases are present?

Cells contain a mixture of proteases, but the following generalizations can be made (North, 1989):

- ▶ Serine proteases are widely distributed in most types of cells.

- ▶ Bacterial extracts typically contain serine and metalloproteases.
- ▶ Extracts from animal tissues contain mainly serine, cysteine and metalloproteases. Some also contain aspartic proteases.
- ▶ Plant extracts contain large amounts of serine and cysteine proteases.

What inhibitor is best to use?

Each different protease inhibitor will generally inhibit only one or two types of proteases (Table 4B.1). Thus, we recommend adding more than one inhibitor to any cell extract, since multiple inhibitors provide better protection.

Note: Boehringer Mannheim offers a full line of protease inhibitors, including all those listed in Table 4B.1. See Section 5B of this manual for ordering information.

Prepare a custom “cocktail” of inhibitors to combat the proteases most likely to be present in a given extract. Or, use a commercially available protease inhibitor cocktail such as Complete tablets that can inhibit a wide spectrum of common proteases (Table 4B.1).

However, if you are working with biological material containing considerable amounts of “atypical” proteases, that are not inhibited by a typical inhibitor cocktail, supplement the cocktail with additional inhibitors.

For instance, aspartic proteases (“acid proteases”) are active only at acid pH. If a procedure requires steps (such as elution of tagged proteins from an affinity column) at low pH, add an aspartic protease inhibitor such as pepstatin to the buffers for those steps.

Preparation of cell lysate

No single cell lysis procedure will break open every type of cell and successfully release every tagged protein in a usable form. Lysis procedures must be optimized to ensure maximal cell disruption with minimal damage to the tagged protein.

Inhibitor	Effective against	Working concentration
Complete™ tablets	Serine-, cysteine-, and metalloproteases, calpains	1 tablet for 25–50 ml
APMSF	Serine proteases	10–40 µg/ml (10–20 µM)
Aprotinin	Serine proteases	0.06–2.0 µg/ml (0.01–0.3 µM)
E-64	Cysteine proteases	0.5–10 mg/ml (0.14–28.0 µM)
EDTA	Metalloproteases	500–5000 µM
Pefabloc® SC	Serine proteases	100–1000 µg/ml (400–4000 µM)
Pepstatin	Aspartic (acid) proteases	0.7 µg/ml (1 µM)
PMSF	Serine and cysteine proteases	17–170 µg/ml (100–1000 µM)
Leupeptin	Serine and cysteine proteases	1–10 µg/ml (2–20 µM)

Table 4B.1: Specificity of protease inhibitors