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.

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

Table 4B.1: Specificity of protease inhibitors
A number of different lysis buffers can be used to release tagged proteins from cells. In general, the conditions used for lysis should be as gentle as possible to retain the antibody binding sites and to avoid solubilizing background proteins, but harsh enough to ensure quantitative release of the antigen. Variables that can drastically affect the recovery of protein of interest include salt concentration, type of detergent, presence of divalent cations, and pH.

The most commonly used lysis buffers include:
- NP-40 lysis: 150 mM NaCl, 1.0% NP-40, 50 mM Tris (pH 8.0)
- High salt lysis: 500 mM NaCl, 1.0% NP-40, 50 mM Tris (pH 8.0)
- Low salt lysis: 1.0% NP-40, 50 mM Tris (pH 8.0)
- RIPA: 150 mM NaCl, 1.0% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris (pH 8.0)

Cells can be lysed by several techniques depending on the cell type.

For cells grown in tissue culture, the most frequently used method of lysis is treatment with detergents. Cell membranes can also be broken by physical shearing using a dounce homogenizer, passing through a needle, or sonication. Of the methods for lysis of tissue culture cells, the freeze-thaw lysis is the least desirable.

For yeast, the preferred method of lysis is mechanical shearing either by vortexing the cells in the presence of glass beads or by sonication. Enzymatic degradation of the cell wall is one effective method of producing spheroplasts, which can then be lysed with detergents.

For the preparation of bacterial lysates, sonication, which breaks the cell wall and membranes, is used most often. Another common method is lysozyme degradation of the cell wall followed by lysis with detergents.

For information on the factors that can influence successful cell lysis, consult the literature (Sambrook, Fritsch, and Maniatis, 1989, p. 18.30–18.33; Harlow and Lane, 1988). For information on lysis procedures for various types of cells, see Table 4B.2.

### Table 4B.2: Lysis procedures for different types of cells.

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Lysis method</th>
<th>Suitable for analysis by*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (E. coli)</td>
<td>Detergent</td>
<td>WB</td>
<td>Sambrook et al. (1989, p. 18.40)</td>
</tr>
<tr>
<td>Yeast (S. cerevisiae)</td>
<td>Mechanical disruption, Spheroplasting and detergent</td>
<td>IP, WB</td>
<td>Sambrook et al. (1989, p. 18.35; 18.38); Shen, et al. (1993)</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>Detergent</td>
<td>IP, WB</td>
<td>Algrain et al. (1993); Dietzen et al. (1995); Sambrook et al. (1989, p. 18.32; 18.62)</td>
</tr>
<tr>
<td>Spheroplasting</td>
<td>AC, IP, WB</td>
<td></td>
<td>Wadzinski et al. (1992)</td>
</tr>
<tr>
<td>Sf9 insect cells</td>
<td>Homogenization</td>
<td>WB</td>
<td>Gimpl et al. (1995)</td>
</tr>
</tbody>
</table>

* Abbreviations: AC, affinity chromatography; IP, immunoprecipitation; WB, Western blotting

Note: These are a mere sampling of available lysis procedures and are not necessarily the best or the most appropriate procedure to use in a given experimental system.

### References


