Protein Sample Preparation

Protocol

Bulletin 6198

Microbial Cultures

Use the MicroRotofor™ cell lysis kit (bacteria) or the MicroRotofor cell lysis kit (yeast). Alternatively, use the protocol detailed here, which relies on cell lysis with ultrasonic waves in combination with a solubilization in SDS under elevated temperature. This ensures deactivation and denaturation of proteases.

1. Centrifuge cells (~5 × 10^7) for 3 min at 5,000 × g and resuspend the pellet in an equal volume of 37°C PBS and centrifuge again. Repeat two more times to remove all interfering material (extracellular proteases and growth media).
2. Add 200 µl of hot (95°C) SDS sample solubilization buffer to the pellet and vortex thoroughly.
3. Sonicate the sample solution 10 times for 1 sec each at ~60 W and ~20 kHz. Incubate at 95°C for 5 min.
4. Cool the sample to 20°C and dilute with ~250 µl 2× SDS-PAGE sample buffer. Incubate for another 20 min at room temperature.
5. Centrifuge the sample solution at 20°C for 30 min at 14,000 × g and harvest the supernatant.
6. Perform the protein assay. The protein concentration should be ~5 µg/µl.

Protein Fractions from Chromatography

When checking fraction purity or the enrichment of a particular protein after a chromatographic separation, you can observe the presence of high concentrations of salt, detergent, denaturants, and organic solvents. For example, in ion exchange chromatography, proteins are eluted by a salt gradient. But, the salt concentration of the corresponding fractions can be as high as 0.5 M, a concentration that interferes with SDS-PAGE.

Remove salt and other contaminants by one of the following approaches:

- Buffer exchange — use Bio-Spin® or Micro Bio-Spin™ columns, which are filled with size exclusion media equilibrated in Tris buffer. These columns accommodate a sample volume 50–100 µl and remove compounds <6 kD within 10 min. Mix the purified sample with 2× SDS-PAGE sample buffer
- Precipitation — use the ReadyPrep™ 2-D cleanup kit (based on an acetone/TCA preciptation) for simultaneous removal of interfering substances and concentration of dilute samples (<50 ng/ml)

TIPS

Reproducible lysis and protein solubilization of bacteria and yeast is challenging because the cells may release proteases and other enzymes into the growth medium. Wash the cultures thoroughly with isotonic buffers and take precautions to inactivate the proteolytic activity after cell lysis. Extensive disruption of microbial cells is required, usually with the help of a French press, bead impact instruments, or sonicator

REAGENTS

- SDS sample solubilization buffer
- SDS-PAGE sample buffer (2x)
- Phosphate buffered saline (PBS)

EQUIPMENT

- Centrifuge
- Sonicator

PRODUCT LINKS

- MicroRotofor cell lysis kit (bacteria)
- MicroRotofor cell lysis kit (yeast)
- *RC DC* protein assay
- SDS-PAGE sample buffer (2×)
- Bio-Spin Columns
- Micro Bio-Spin Columns
- ReadyPrep 2-D cleanup kit

BIO-RAD

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).

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