

Preparation of cleared lysates - Sonicator

Aim:

- Cell lysis and release of protein of interest

Timeframe

- 60 minutes

Materials:

- Ice
- Lysozyme
- Sonicator
- Benzonase® Nuclease

Procedure:

1. Centrifuge cell cultures at 5000g rpm for 10 minutes, decant supernatant and store cell pellet until use in -20 freezer.
2. Thaw the cell pellet for 15 min on ice and resuspend the cells in 5 ml of purification buffer (1 M Tris-Cl 1.5 M NaCl 10 mM EDTA)
3. Use a sonicator equipped with a microtip. Sonicate on ice. Use fifteen 30 s bursts at 200–300 W with a 30 s cooling period between each burst.
4. (Recommended) If the lysate is very viscous, add benzonase (3 U per ml culture volume) and incubate on ice for 10–15 min. Alternatively, draw the lysate through a blunt-ended, narrow-gauge syringe needle several times.