Protein renaturation from pellet samples

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Abstract

After cell lysis and centrifugation the possibly misfolded proteins can be refolded with this protocol.

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Protocol

Step 1.
Resuspend the samples in 50 mM Tris-HCl at pH 7.4, 4 M urea

Step 2.
Sonicate samples for four minutes, 5 sec on, 5 sec off, at 30 % Amp to wash

Step 3.
Centrifuge 12 000 g for 10 min, RT

Step 4.
Collect supernatant

Step 5.
Repeat the washing three times, collect supernatant

Step 6.
Incubate the supernatant at 37 °C for minimum 1 hour

Step 7.
If your sample is cloudy after this add 8 M urea until it becomes clear, incubate for 1 h at 37 °C

Step 8.
Dialyze your denaturated protein samples first at a 1:10 ratio against buffer containing 10 % v/v glycerol and 0.1 mM EDTA (refolding buffer), 20 mM HEPES pH 7.4 at 4 °C for 4 h

Step 9.
Optional: Concentrate your sample

Step 10.
Second dialysis with a 1:100 ratio against the same buffer at 4 °C for 16 h or O/N

Step 11.
Concentrate samples for further analysis