Detailed Protocols
Protocol 1. Cell lysis under native conditions

Note: The use of buffers containing **0.05% Tween 20** throughout the purification procedure is strongly recommended in order to optimize the separation characteristics of the magnetic beads. This is especially important when performing 96-well procedures.

Purification can also be carried out without clearing the lysates, but care should be taken that the lysate is not too concentrated. The volume of the crude, uncleared lysate should not be more than 1/5 to 1/10 of the culture volume (e.g., lyse cells from 1 ml culture in 0.2 ml or at the very least 0.1 ml lysis buffer). DNA, which can disturb the separation of the beads, should be eliminated by DNase treatment.

Materials

Bacterial pellets (For guidelines on cell cultivation see appendix, page 80.)

Lysis Buffer-Tween for native conditions (containing Tween 20)

For buffer and reagent compositions, see appendix, page 80.

Procedure

1. **Thaw cells for 15 min and resuspend in Lysis Buffer-Tween.**

   For 1 ml cultures grown in 96-well blocks, 200 µl Lysis Buffer-Tween should be used for each well. In 96-well blocks it is not practical to lyse the cells by sonication. **Freeze the cell pellets** at –20°C or –70°C for at least one hour after harvesting — without freezing yields obtained without sonication are reduced by up to 75%.

   For larger culture volumes, increase the lysis volume appropriately.

   Adding 10 mM **imidazole**, inhibits binding of nontagged proteins. If the tagged protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM.

2. **Add lysozyme to 1 mg/ml.**

   If uncleared lysate will be used for processing add DNase (e.g., Benzonase® at 1 U/ml culture volume)

3. **Incubate on ice for 30 min.**

   Alternatively, when using 96-well blocks, incubation can be at room temperature for 15 min.

   Generally, we recommend using cleared lysates for binding to Ni-NTA Magnetic Agarose Beads, but it may be possible to obtain good results by using crude lysates without clearing them. In this case, use dilute lysates with a volume of between 1/5 and 1/10 of the original culture volume (e.g., lyse cells from 1 ml culture in 0.2 ml or at the very least 0.1 ml Lysis Buffer-Tween), add RNase A to 10 µg/ml and DNase I to 5 µg/ml, and incubate on ice for 10–15 min.

4. **Sonicate or homogenize on ice to lyse cells (6 times for 10 s each time with 5 s pauses between).**

   When using 96-well blocks, cover block with tape and vortex 6 times for 5 s each time on a low to medium setting. This step may need to be optimized for different cell cultures and vortexers.

   If uncleared lysate is being used for processing do not carry out step 5 but proceed directly with downstream application.

5. **Clear lysate by centrifugation at 10,000 x *g* for 30 min at 4°C. Collect supernatant.**

   Save 20 µl of the cleared lysate for SDS-PAGE analysis.

   Note: 96-well blocks can be centrifuged at 5000 x *g* for 30 min. Centrifuge 4-15 fitted with the Plate Rotor 2 x 96 is ideal for centrifugation of the 96-well blocks (for more information, call one of the QIAGEN Technical Service Departments).