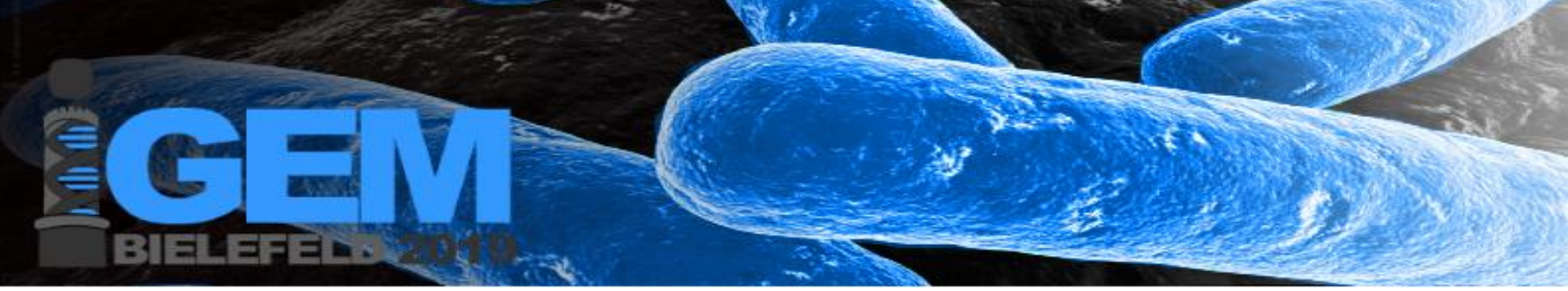

Purification of polyhistidine-tagged proteins from E. coli under native conditions with Protino® Ni-TED 1000 Packed Columns from Macherey Nagel

1. **Preparation of buffers for purification under native conditions**
    - Dilute the contained LEW/Elution Buffer stock solutions of the Protino® Ni-TED 1000 Packed Columns according to the given instructions.
      Note: Lysis buffer, equilibration buffer, and washing buffer are the same.

2. **Preparation of cleared lysates under native conditions**
    - Thaw the cell pellet from an *E. coli* expression culture on ice (if frozen). Resuspend **1 g of pelleted, wet cells in 2 – 5 mL LEW Buffer**. Pipette up and down, or use stirring until complete resuspension without visible cell aggregates. Perform this step on ice.
    - Add **lysozyme** to a final concentration of 1 mg/mL. Stir the solution **on ice for 30 min**.
    - Sonicate the suspension on ice according o the instructions provided by the manufacturer (e.g., use 10 x 15 bursts with a 15 s cooling period between each burst).

3. **Purification with Protino® Ni-TED 1000 packed columns**
    - Cultivate and harvest cells through centrifugation at 4,500 – 6,000 *x g* for 15 min at 4°C. remove supernatant and store cell pellet a -20°C if not processed immediately.
    - Prepare a working solution (for each column) containing the following components: 1.5 mL 8 x LEW Buffer, 10.5 mL water, which makes up a whole 1 x LEW Buffer with 12 mL volume (dilution).
    - Prepare the cell extract accordingly.
    - Add 2 mL 1 x LEW Buffer to the column for equilibration. Allow the column to drain by gravity.
    - Load the clarified lysate onto the pre-equilibrated column for binding and allow the column to drain by gravity.
    - Wash with 2 x 2 mL 1 x LEW Buffer. Allow the column to drain by gravity.
    - Add 3 x 1.5 mL of 1 x Elution Buffer for the elution. Allow the column to drain by gravity.

From: iGEM Bielefeld-CeBiTec

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