IMPACT™ Kit: Protein Purification from NEB

1. **Bacteria Cultivation:**
   - Inoculate an overnight culture with 25 mL LB and the required antibiotic in a 250 mL culture flask. Incubate at 37°C.
   - Start a new culture with 250 mL LB and the required antibiotic in a 1 L culture flask from the overnight culture with start-OD_{600} = 0.1
   - Cultivate at 37°C until the OD-value reaches 0.5 – 0.8.
   - Initiate expression by adding 0.4 M IPTG and let the bacterial growth proceed at 37°C for 30 minutes.
   - Expression of the protein takes place at 17°C overnight. Use shaker in cooling chamber.
   - Harvest the cells in a centrifuge with cooling functions for 20 minutes on 4,500 rpm at 4°C in a 250 mL centrifuge container.
   - Decant the supernatant and resuspend in 35 mL lysis buffer.
   - Transfer the resuspension in a 50 mL tube with screw cap.

2. **Cell Disruption:**
   - The cells are to be disrupted in lysis buffer in a French Press® with the pressure of 1,212 Psi.
   - Repeat the disruption 2 – 3 times.
   - Centrifuge the cells for 1 hour on 4,500 rpm and 4°C. transfer the supernatant into a new 50 mL tube.

3. **Column preparation:**
   - Provide a rubber hose and a clamp to the bottom part of the column.
   - Fill the column with 10 mL chitin-beads.
   - Wait until the beads drop to the bottom and add the filter to the column material.
   - Remove ethanol through the filter.
   - Equilibrate the column with 10x column buffer.

4. **Loading the column:**
   - Load the column with the supernatant from the cell disruption (max. 0.5 – 1.0 mL/min)
   - Wash the column with 10x column-wash-buffer

5. **Cleavage Initiation:**
   - Provide fresh cleavage buffer under no-light conditions (cover with tinfoil)
   - Run 15 mL of the cleavage buffer through the column.
   - Let sit at room temperature in the dark for 20 – 24 hours.

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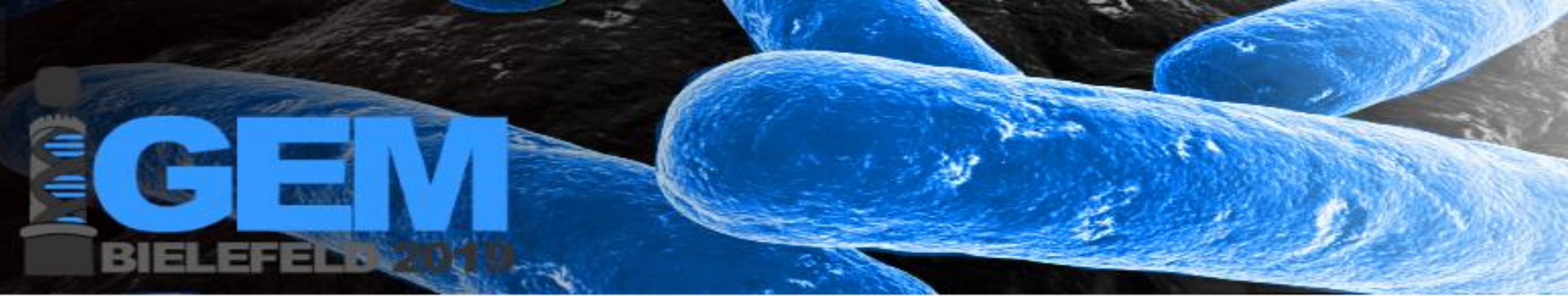

6. **Elution:**
    - After the cleavage, elute with 1x column buffer.
    - Concentrate the protein solution with the suitable protein filter.
    - Wash the protein 3 times each with 10 mL column buffer.
    - The end volume of the eluat: 250 – 500 µL
    - Create a 1:1 mixture of the eluat with 86% glycerin and store at -20°C.

Lysis Buffer:
- Tris 25 mM
- NaCl 500 mM
- EDTA 1 mM
- Triton X-100 0.15%
- PMSF 20 µM
- TCEP 1mM

Adjust pH to 8.5

Column Buffer:
- Tris 25 mM
- NaCl 500 mM
- EDTA 1 mM

Adjust pH to 8.5

Column-Wash-Buffer:
- Tris 25 mM
- NaCl 1500 mM
- EDTA 1mM

Adjust pH to 8.5

Cleavage Buffer:
- Tris 25 mM
- NaCl 500 mM
- EDTA 1 mM
- DTT 50 mM

Adjust pH to 8.5

From: iGEM Bielefeld-CeBiTec

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