

## Purification of mCherry and Cell Lysis with Ribolyser

### 1. Sample preparation

- Determine the weight of the cell pellet(s) obtained from cultivation.  
Our samples:
  - pTXB1\_mCherry: 1.92 g
  - pSB1C3\_mCherryHis: 1.63 g
- Compose 35 ml of lysis buffer (see below).
- For a His-purification, take 12  $\mu$ l (see the protocol for IMPACT kit).
- Resuspend the pellets in 4 ml LEW buffer (pSB1C3\_mCherryHis) and 35 ml lysis buffer (pTXB1\_mCherry).

### 2. Cell Lysis

The cell lysis is carried out in a ribolyzer with the following conditions:

- Metal beads for the frictions to the cell walls which would lead to the disruption of the cell wall.
- 3 rounds each time lasting 30 seconds.
- 8000 rpm
- 4°C

### 3. Centrifugation

Centrifuge the lysed cells at 4500 rpm and 4°C for 1 hour.

### 4. Purification

Proceed the purification using IMPACT™ Kit (see the protocol for the kit).

#### Lysis Buffer:

- Tris 25 mM
- NaCl 500 mM
- EDTA 1 mM
- Triton X-100 0.15%
- PMSF 20  $\mu$ M
- TCEP 1mM

Adjust pH to 8.5

From: iGEM Bielefeld-CeBiTec