# Preparation of cell lysates from *E. coli* by sonication

## Materials

Lysis buffer

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| 50 mM Tris-HCl pH 7.5 |
| 50-200 mM NaCl* |
| 5 mM DTT |
| 1 mM PMSF |

* The NaCl concentration used in the lysis buffer depends fully on the application. In case of affinity chromatography on a Ni-column the NaCl concentration is usually 200 mM but when the first purification step is ion exchange chromatography **no** salt should be added.

Stock solution

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| 100 mM PMSF (phenylmethylsulfonyl fluoride) in isopropanol |

## Procedure

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| **1.** | Resuspend the cells in chilled lysis buffer. Normally ratios of cell wet weight to buffer volume of 1:1 to 1:4 are used. |
| **2.** | Cool the cell suspension on ice for 10 min.<br>✍ Add 10 µl PMSF (100 mM) per ml of celsuspension after this step. |
| **3.** | Sonicate the cell suspension with 10 short burst of 10 sec followed by intervals of 30 sec for cooling.<br>✍ Keep the suspension at all times on ice.<br>✍ Avoid foaming.<br>✍ Don’t go away while the sonicator is in operation. It is possible that the beaker breaks or turns in the melting ice. |
| **4.** | Remove cell debris by ultracentrifugation at 4°C for 30 min at 45 000 rpm using a 45Ti rotor (Beckman). |