Preparation of cell lysates from *E. coli* by enzymatic lysis

Materials

Chemicals

lysosyme

Lysis buffer

50 mM Tris-HCl pH 7.5 50-200 mM NaCl* 5% glycerol (v/v) 1 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 solutions

1 mg/ml DNase and in water
100 mM PMSF (phenylmethylsulfonyl fluoride) in isopropanol
1M $MgCl_2$

Procedure

1.	Resuspend the cells in chilled lysis buffer in a ratio of 1 g cell wet weight to 1 ml lysis buffer. ✍ Add the PMSF (10 µl PMSF (100 mM) per ml of celsuspension) at this point.
2.	Add lysosyme to a final concentration of 300 µg/ml and incubate the cell suspension at 4°C for 4 h.
3.	Add 5 µl $MgCl_2$ (1 M) and 1 µl DNase solution (1 mg/ml) per ml of cell suspension and incubate the solution at 4°C for 30 min.

4.	Remove cell debris by ultracentrifugation at 4°C for 30 min at 45 000 rpm using a 45Ti rotor (Beckman).