Galactose induction for analytical purposes

- solutions:
  - 5x buffer A: 0.1 M Tris pH 7.5, 5 mM EDTA, 50% glycerol
  - extraction buffer: 1x buffer A, 150 mM NaCl, (0.2 mM PMSF [toxic!])
  - basic medium:

0.67% yeast nitrogen base w/o amino acids	6.7 g
amino acid drop out mix	0.87 g
2% (w/v) sodium lactate	25.6 ml 60%(w/w)
3% glycerol	30 ml
add H_2O to 1 l	

- preculture in 5 ml SD medium, incubate 24 h at 30°C
- start main culture in 50 ml basic medium at OD_{600}=~0.2
- incubate ~ 16h at 30°C (OD_{600} should reach 1-2)
- add 2% galactose (5 ml 20%)
- induce for 6 h at 30°C
- spin down at ~4000 rpm for 10 min
- resuspend pellet in 1 ml extraction buffer and transfer to screw cap tube for cell disruption
- spin down in table top centrifuge
- pellet can be frozen in liquid N_2 and stored at -80°C

Protein extraction

- material:
  - acid washed glass beads (Ø=0.45 mm):
  - leave 15 min in 5 M nitric acid (HNO_3)
  - wash with H_2O until pH is neutral (check with pH paper)

- add 250 µl extraction buffer to cells (make sure it contains PMSF)
- add 250 µl acid washed glass beads
- extract proteins in bead beater (fast prep) for 1-2x 45 sec (setting 4)
- spin down 5-10 min at 4°C (eppendorf centrifuge)
- transfer supernatant to fresh tube (~200 µl)
- measure protein concentration with Bradford assay (see Bio-Rad protein kit)
- store extracts at -80°C