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 H₂O to 1 l

- **preculture** in 5 ml SD medium, incubate 24 h at 30°C
- **start main culture** in 50 ml basic medium at OD₆₀₀≈0.2
- **incubate** ~ 16h at 30°C (OD₆₀₀ 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 N₂ and stored at -80°C

**Protein extraction**

- **material:**
  - acid washed glass beads (Ø=0.45 mm):
    - leave 15 min in 5 M nitric acid (HNO₃)
    - wash with H₂O 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