Yeast cell lysis

Introduction

Protocol from Knop et. al. 1999 (YEAST vol. 15, 963 - 972), used by Bakkaiova et. al. 2014 (Eukaryotic cell vol. 13 no. 9 p. 1143 - 1157) to lyse *Y. lipolytica.*

Materials

- Cold MQ
- Lysis buffer
  - 1.85M NaOH
  - 7.5% β-mercaptoethanol
- 55%w/v trichloroacetic acid (TCA) (stored in the dark)
- HU-buffer (stored at -20°C without DTT)
  - 8M urea
  - 5% SDS
  - 200mM Tris pH 6.8
  - 1mM EDTA
  - Bromophenol blue
  - 1.5% DTT
- Pre cooled centrifuge (4°C)

Procedure

Harvest cells

1. Grow cells in desired growth phase to OD600 0.5 - 3.0
2. Extract 1mL broth
3. Spin cells for 5 min @ 3000g
4. Resuspend cells in 1mL cold MQ

Cell lysis

5. Mix cell suspension with 150µL Lysis buffer
6. Incubate on ice for 15min

00:15:00

7. Add 150µL 55% TCA
8. Incubate on ice for 10 min

00:10:00

Purify crude protein extract

9. Spin for 10 min @ 14.000RPM, preferably @4°C
10. Remove supernatant
11. Spin briefly
12. Remove all residual traces of TCA by pipetting and aspiration
13. Add 100µL HU-buffer per OD600 of cells which where extracted in step 2
14. Resuspend proteins (Resuspension can be aided by using a solicitor bath)
15. Incubate solutions for 10 min @ 65°C preferably in a thermoshaker

00:10:00

16. If a yellow colour develops, add 1–3 µL of 2M Tris-base
17. Spin solutions for 5 min @ 14.000RPM
18. Use supernatant for SDS page