Denatured yeast protein extracts by NaOH lysis/TCA precipitation

05-13-06, Sigurd Braun; according to Knop et al. 1999, Yeast 15(10B):963-72

use:
for quick preparation of protein samples (avoid of glass beads and/or phenol and/or boiling)
time: less than 60 minutes

Buffers/Solution:

- **NaOH/BME *(Prep under hood fresh each time):*** 138.75 µl 2N NaOH + 11.25 µl BME per sample

  (usually, for 12 samples a mix of 1850 µl NaOH + 150 µl BME is prepared!)
- **TCA:** 55% (w/v) trichloroacetic acid in H2O (stored at 4 deg) – *make as follows ; 30ml H20 + then 55ml OF 100% TCA - + then 15ml H20 (don't add water to acids – or "don't give acids a drink")*
- **HU buffer:** 200 mM phosphate buffer, pH 6.8, 8 M urea, 5% w/v SDS, 1 mM EDTA, 100 mM DTT, bromophenol blue; stored at –20 deg w/o DTT; **DTT is added freshly from 1 M stock).** *(add 80ul of DTT (1M) to 750ul of HU)*

cell lysis and protein precipitation:

1. harvest yeast cells corresponding to an OD_{600} = 1 *(inoculate from plate with toothpick into 3ml tube - minimal media for S Cere – grow overnight – for S Cere – then measure OD and inoculate into 4ml – (400 x .15/OD) – and grow for 5-6 hours until an OD 0.35-0.50 as measured at a 500ul:500ul dilution= thus 0.6-1 real). You want 1ml with an OD of 1 = so if its below an OD of 1 - then 1000ul/0.6 (for example) OD or above = 1666ul (pipette 833ul twice) – then spin down the pellet and remove supernatant – You can freeze them at -80 here and resume the next day).*
2. resuspend cell pellet in 1 ml ice-cold water
3. add 150 µl NaOH/BME to each sample
4. incubate on ice with occasional vortexing for 15 min
5. add 150 µl TCA to each sample
6. incubate on ice with occasional vortexing for 15 min
7. spin down in a table centrifuge at 4 deg at max. speed (e.g. 20K) for 15 min
8. take off most of supernatant with pipette or with vacuum pump tip/syringe needle
9. spin at max. speed for 5 min, discard rest of SN
10. add 100 µL ice cooled aceton (flick with finger)
11. spin down at max. speed 15 min., discard SN

12. spin at max. speed for 5 min carefully take off remaining supernatant with precaution (protein pellet becomes slippery after a while)
13. resuspend pellet in 33 µl HU buffer+DTT (30µL HU + 3µL DTT), vortex with platform-vortexer 3-5 min

14. optional: when buffer turns yellow due to some residual TCA in the resolved protein pellet, add some (10-20) µl of 1 M Tris pH 6.8

sample denaturation/SDS PAGE loading:

1. heat denature at 65-70 deg for 10-15 min
   (do NOT boil as urea denatures/aggregates at higher temperatures than 70 deg)
2. spin briefly in table centrifuge (RT, 12K, 1 min)
3. load 5-10 µl of sample (corresponds to 0.1 – 0.2 OD_{600} of yeast cells)