Denatured yeast protein extracts by NaOH lysis/TCA precipitation

according to Knop et al. 1999, *Yeast* 15(10B):963-72
modified by Sigurd Braun

use:
quick preparation of protein samples from *S. cerevisiea, S. pombe, C. neoformans*
(avoidance of glass bead disruption/phenol/boiling)
time: less than 60 minutes

Buffers/Solution:

- **NaOH/BME:** 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)
- **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)

cell lysis and protein precipitation:

1. harvest yeast cells corresponding to an OD_{600} = 1-2
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
7. spin down in a table centrifuge at 4 deg at max. speed (e.g. 20K) for 20 min
8. take off most of supernatant with pipette or with vacuum pump tip/syringe needle
9. spin again briefly at max. speed for 1 min
10. carefully take off remaining supernatant with precaution
    (protein pellet may be loose)
11. resuspend pellet in 50 µl HU buffer
12. 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

alternatively, after taking off supernatant (step 4), add 100 µl of chilled acetone (–20 deg) to remove any residual TCA; continue with step 5
(only recommended when protein amounts are precipitated corresponding to more than 1 OD_{600} of yeast cells)

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)