Preparation of S. pombe protein samples for Western Blot (Urea Method)

Reagents:
8M Urea LB = 2x SDS-PAGE Loading Buffer w/ 8M Urea
or 8M Urea LB w/o 2-ME and BPB (for protein assay)
Stop Buffer (4˚C)
Glass beads (4˚C)

1) Culture cells in 50 ml (10ml just to briefly check protein level) YES medium @ 30˚C for O/N.

2) If cell density is more than 1.5 OD/ml, dilute cells in YES at 0.4 OD/ml, culture cells another 2 to 3 hr.

3) Collect cells by centrifugation. (2400 rpm, 4 min, 4˚C)
*Optical cell density should be 0.5~1.5 OD/ml

4) Decant medium, re-suspend cells with 10 ml Stop Buffer, and collect cells by centrifugation (2400 rpm, 4 min, 4˚C)

5) Decant buffer, and re-suspend cell pellet in 1ml Stop Buffer. Aliquots cell suspension to two 1.5 ml screw-top tubes (if from 10ml culture, 1 tube). Centrifuge to collect cells. (7000 rpm, 15 sec, 4˚C)

6) Remove sup by an aspirator, re-suspend cells in 100 μl of Stop Buffer, and vortex to mix thoroughly.

7) Boil cell suspension for 5min, and cool it down on ice.
*Cell suspension can be snap-frozen in liquid nitrogen (or EtOH-dry ice) and kept at -80˚C

8) Add 120 µl 8M Urea LB. Add glass beads until over the surface of liquid.
*If protein assay is required, 8M Urea LB **w/o** BPB & 2-ME should be used.

9) Fast Prep. (Speed: 6.0, Time: 20 sec x 2, 2 min interval)

10) Pierce the bottom of the tube with a heated needle (25G). Place the tube on top of a new 1.5 ml tube. Centrifuge. (3000 rpm, 30 sec, RT) The protein extract can be collected in the bottom tube.

11) ONLY WHEN PROTEIN ASSAY NEEDS TO BE DONE: *If protein assay is required, centrifuge the sample (the bottom tub) at 13krpm for 10min at 4˚C. Keep the tube on ice. Take 10 ul of the sup, and add it to 500 ul of BCA solution for protein assay (see BCA instruction). While you are incubating BCA samples at 37˚C, add 10 μl each of "3xSDS LB w/ thick BPB" and "2-ME" to the tube.

12) Vortex the tube to mix the content well. Boil 5 min. On ice 5 min. Keep the tube at -20˚C.

13) ONLY WHEN PROTEIN ASSAY NEEDS TO BE DONE: *If protein assay was done, adjust the protein concentration with 1xSDS LB w/ 4M Urea.

14) To apply the sample to SDS-PAGE, mix the sample well by vortex. If precipitation occurs, warm the sample at 37˚C briefly. Centrifuge to collect all liquid at the bottom of the tube. At this point, precipitation can be ignored. The supernatant is the protein extract. Apply 5 to 10 µl of the protein extract for each lane.