Yeast Whole Cell Extracts

1. Grow yeast in selective media (if needed) to saturation overnight (5 mL).

2. Back-dilute into 10 mL of YPD to 0.25 OD\textsubscript{600}/mL and grow for 4-5 hours at 30°C until exponential phase is reached (1.0 OD\textsubscript{600} approximately).

3. Spin down cells for 5 minutes in clinical centrifuge.

4. Pour off media. Resuspend cells in 1 mL of water, gently.

5. Spin down at 6000 rpms for 1 minute.

6. Pour off water and pipette off excess.

7. Resuspend into 250 uL of “Thorner Buffer”.

8. Add approximately 50-100 uL of glass beads (0.5 mm) to tube.

9. Spin down for 3 seconds at 3000 rpms (bring beads to bottom of tubes).

10. Vortex on multi-vortexer for 8-9 minutes at max setting.

11. Spin down yeast, centrifuge at maximum for 5 minutes.

12. Carefully transfer (only) the supernatant to a fresh tube. (Usually is between 100-150 uL total volume left now. Do not transfer any cell debris or pellet).

13. Thorner buffer can have (or not) Bromophenol blue added (if protein assay is to be run, no blue should be present).

14. Freeze overnight at -20°C.

15. On day of SDS-PAGE/Western blot, add 5 uL of BME (to 95 uL of Thorner Buffer + sample) for final concentration of 5%.

16. Mix with Laemmli Sample Buffer (2x or 4x stock) for final 1x concentration and mix.

17. Boil for 5 minutes and spin at maximum for 5 minute. Load onto SDS-PAGE gel.

**Thorner Buffer (10 mL of 1x Buffer)**

8 M urea (4.8g urea)  
5% SDS (2.5 mL of 20% SDS)  
50 mM Tris-HCl pH 6.8 (0.5 mL of 1 M Tris-HCl pH 6.8)  
3.4 mL Water