Yeast mini whole cell extract using glass beads for western analysis

Before trying this method, try rapid protein prep which involves boiling yeast in SDS PAGE buffer

1. Grow 100 ml yeast to A600 ~1.0. If the yeast are overgrown, use proportionally less cells for the prep.

2. Collect cells by centrifugation and wash in 20 ml cold extraction buffer (containing DTT and protease inhibitors). Keep everything cold from this point on.

3. Resuspend cells in 600 microliters extraction buffer and transfer to 13 x 100 mm glass test tubes on ice. Add 400 microliters glass beads.

4. In the cold room, vortex for 1 min on ice and keep on ice for at least 1 min while vortexing other tubes.

5. Repeat the vortexing step (step 4) for a total of 5 times.

6. Spin cells in pre cooled clinical centrifuge for 3 min at top speed.

7. Using a 1 ml pipetman, remove the supernatant, leaving most of the glass beads behind (transferring some of the yeast is OK). Transfer to a microcentrifuge tube.

8. In the cold room, spin at top speed in the microfuge for 30 min. Remove supernatant to a new tube being careful not to carry over any cell debris.

9. Measure protein concentration using BioRad assay and freeze in aliquots. Load 20-40 micrograms on protein gel for western analysis.

Extraction Buffer:
200 mM Tris pH 8.0
150 mM Ammonium sulfate
10% glycerol
1 mM EDTA

Add fresh Protease inhibitors on day of extract preparation: