Improved Yeast Whole Cell Extract Methods

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SDS PAGE Sample Buffer:	**10 ml**
0.06M Tris-HCl, pH 6.8	0.6 ml 1M Tris 6.8
10% (v/v) glycerol	2 ml 50% glycerol
2% (w/v) SDS	2 ml 10% SDS
5% (v/v) 2-mercaptoethanol	0.5 ml 2-mercaptoethanol
0.0025% (w/v) bromophenol blue	0.2ml Sat. Bromophenol blue

Can store buffer frozen at —20 degrees for ~ 6 months.

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1. Grow yeast cells to log phase (~1 x 10^7 cells/ml; A600 = 0.7) and collect 1.0 - 1.5 ml cells in 1.5 ml microfuge tube (adjust volumes according to cell density of cultures). Spin 1 minute, 14,000 x g.

For cell growth in synthetic media, 10 microliters of a saturated YPD overnight culture inoculated to 5 ml SD + essential amino acids for ~16 hrs gives A600 of 0.5 to 1.0 for wild-type cells @30 degrees.

or: 150 microliters of saturated YPD culture diluted to 5 ml YPD and grown for ~5 hrs at 30 degrees gives an A600 of ~0.8 for wild-type cells.

2. Resuspend cell pellets in 200 microliters 0.1 M NaOH
Incubate 5 min room temp.

3. Spin cells, remove NaOH and resuspend in 100 microliters SDS PAGE sample buffer.

4. Heat 95 deg for 5 min. Spin 5 min in microfuge (important to heat cells before storage).

Can run immediately on gel, or store samples frozen at -70 deg.

The NaOH treatment seems to work well to solubilize proteins that are not released by boiling cells in SDS PAGE sample buffer.

An alternative method to that above is to first resuspend cells in 200 microliters of 2M LiOAc for 5 min RT. Spin cells and remove sup. Resuspend in 200 microliters 0.4M NaOH for 5 min RT. Spin and resuspend cells in SDS PAGE buffer as above and heat 5 min 95 deg.

Reference: Zhang et al (2011) Yeast 28:795-798.