**Yeast Whole Cell Extract for use in Western analysis**

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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.

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

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