Glass Bead Prep (Rougher)

Stuff you need:
Sample Buffer:
0.06M Tris-HCl, pH 6.8
10% (v/v) glycerol
2% (w/v) SDS
5% (v/v) 2-mercaptoethanol
0.0025% (w/v) bromophenol blue
0.1 M PMSF
0.5 M Benzamidine

1. Grow 25 ml of cells to mid-log.
2. Spin down (2500 rpm for 5 minutes). Wash 1X with water and spin again.
3. Resuspend in 1 ml of water and transfer to 1.5 ml microfuge tube. Spin down for 5 seconds and pour off water.
4. Resuspend in 0.5 ml of ice cold Sample Buffer with freshly added PMSF (0.5 mM) and benzamidine (0.5 mM).
5. Add glass beads (~0.5 ml).
6. Vortex on high 4X for 45 seconds with 30 seconds on ice in between each mixing.
7. Spin for 5 minutes in microfuge at 4 deg C.
8. Transfer supernatant to a new tube and boil for 5 minutes.

Yeast Cell Lysates

1. Grow cells to 0.3 to 0.5 O.D.(600) (~1E7 cells/ml). Use about 1.0 to 5.0 OD of cells per lysate; if your cells are at 0.5 OD per ml, use 2 ml to give you 1.0 OD of cells in your lysate. Use 2.0 ml eppendorf tubes as the flat bottoms make glass bead lysis easier.
2. Harvest cells by spinning in micro-centrifuge for 2 minutes, decant supernatant.
3. Add 200 µl of SUMEB buffer + protease inhibitors.
4. Add 100 µl of 0.5 mm Acid Washed Glass Beads.
5. Vortex in multivortextor or by hand, 3 X 1 min speed setting 7 on our current machine.
6. Incubate for 10 min at 65°C or whatever temperature is desired.
7. Remove the lysate from the beads with a blue pipette tip to a new 1.5 ml eppendorf tube.
8. Spin 5 minutes to clarify. Remove supernatant to a new 1.5 ml eppendorf tube.
9. Use supernatant directly to load gel or dot blot.
Note that protease inhibitors are added from stocks to give 50 fold dilution. Example: 20 µl of stock per 1ml buffer. Use IMMEDIATELY after adding protease inhibitors.

**SUMEB BUFFER (1% SDS, 8M Urea, 10mM MOPS, pH 6.8, 10mM EDTA, 0.01% bromophenol blue)**

To make 100 ml:
- 1.0ml 1M MOPS, pH6.8 stock solution
- 1.0g SDS, or 10 ml 10% SDS stock solution
- 48.05 g Urea
- 2.0 ml 0.5M EDTA stock solution
- 1.0 ml 1% bromophenol blue

This is used for lysing yeast at pH 6.8. For pH 8 lyses, use the variation of SUMEB with Tris buffer known as SUTEB. Also, bromophenol blue can be left out if dye is not desired, such as using the lysate for immunoprecipitations.

**50X STOCK Protease Inhibitors (store at -20°C)**

**PMSF (87 mg/ml) ([500mM] phenylmethylsulfonyl fluoride)**
To make 30 ml: Dissolve 2.61g PMSF in DMSO to equal a final volume of 30 ml.

**LEUPEPTIN AND PEPSTATIN (5 mg/ml)**
To make 10 ml: Dissolve 50 mg LEUPEPTIN or PEPSTATIN in DMSO to equal a final volume of 10 ml.

**TPCK (5 mg/ml) (tosylphenylalanine chloromethyl ketone)**
To make 10 ml: Dissolve 50 mg TPCK in DMSO to equal a final volume of 10 ml.
* Note: The EDTA in the SUME buffer is also a protease inhibitor.