**Simple, fast yeast protein lysate prep:**

- Grow cells to 0.7 to 1.0 O.D.(600) (~1-2E7 cells/ml). Use about 2.0 to 4.0 OD of cells per lysate; if your cells are at 1.0 OD per ml, use 2 ml to give you 2.0 OD of cells in your lysate. Use 2.0 ml eppendorf tubes as the flat bottoms make glass bead lysis easier.

- Harvest cells by spinning in micro-centrifuge for 2 minutes, decant supernatant.

- Add 200 ul of SUMEB buffer + protease inhibitors.

- Add 100 ul of 0.5 mm Acid Washed Glass Beads.

- Vortex in multivortexer for 5 minutes in cold room.

- Incubate for 10 min at 65C

- Remove the lysate from the beads with a blue pipette tip to a new 1.5 ml eppendorf tube.

- Spin 5 minutes to clarify. Remove supernatant to a new 1.5 ml eppendorf tube.

- Use supernatant directly to load gel or dot blot.

Note that protease inhibitors are added from stocks to give 50 fold dilution.

Example: 20 ul of stock per 1 ml buffer.

Use IMMEDIATELY after adding protease inhibitors.

SUMEB BUFFER

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

Bromophenol blue can be left out if dye is not desired, such as using the lysate for immunopreicipations.

50X STOCK Protease Inhibitors (store at -20oC)

PMSF (87 mg/ml) ([500 mM] phenylmethylsulfonyl fluoride)