

Whole Cell Lysis and TCA Precipitation (Rout Lab, 2006)

This is the method for whole cell lysis and TCA precipitation, used by the Rout lab as of 1998-2005. It is especially effective for samples with a large proportion of protein, so alternate protocols should be used if there is only a small amount of total protein in the original sample. This can be used for yeast or bacterial cells.

1. Pellet cells from culture and resuspend in lysis solution as follows. Transfer to 1.5mL centrifuge tube on ice.
 - a. 10mL log phase or 2mL stationary phase → resuspend in 240uL
 - b. 1mL growth test → resuspend in 50uL
2. Set lysis reaction on ice for 10 minutes.
3. Add 50% Tri-Chloro Acetic Acid (TCA) stored at +4°C in the same volume as lysis solution in Step 1, mix well.
4. Set TCA reaction on ice for 10 minutes.
5. Spin 10 minutes, top speed, at +4°C.
6. Aspirate supernatant, and wash remaining pellet with 90% acetone in more than twice the volumes used in Steps 1 and 2, stored at -20°C. (Eg: If using 240uL, add 500uL Acetone; if using 50uL, add 110uL Acetone.)
7. Incubate at -20°C for at least 20 minutes.
8. Spin 10 minutes, top speed, at +4°C.
9. Aspirate acetone, resuspend pellet in Solution A. (Depending on situation, might want to use anywhere from 25-200uL.)
10. Sonicate to break pellet fully.
11. Add Solution B in an equal volume to the Solution A added in Step 9.
12. Mix and incubate at +95°C for 10 minutes.
13. Spin 2 minutes and load 10uL into a protein gel.

Notes:

- Especially when running a Western, it might be necessary to dilute the sample (eg: 1/100) in Morris Buffer before running.
- If there is a high risk of proteolytic degradation, protease inhibitors should be added to the lysis solution.

Solutions:

1mL Lysis Solution:

- 0.185 mL 10N NaOH
- 0.074 mL B-mercaptoethanol
- 0.741 mL ddH₂O
- 1/100 Solution P

Solution A:

- 0.5M Tris base
- 5% SDS

50 mL Solution B:

- 37.5 mL glycerol
- 12.5 mL water
- 0.96g DTT
- 0.05% bromophenol blue

5mL Solution P:

- 2 mg Pepstatin A
- 90 mg PMSF (beware, toxic!)
- bring to 5mL in Ethanol