

## 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 ddH<sub>2</sub>O
- 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