

Freezing Chlamydomonas Cells in Lysis Buffer for RNA extraction

Solutions:

2x Lysis Buffer (20 ml- 10 samples) – note 2X means that you should not dilute this buffer more than 2-fold with cell suspension.

- 2 ml 1 M Tris-Cl, pH 7.5
- 1.2 ml 5 M NaCl
- 1.2 ml 0.5 M EDTA, pH 8
- 7.2 ml H₂O
- 8.0 ml 10% SDS
- 0.4 ml 4 mg/mL Proteinase K (Kept in the -20°C freezer in 5069 – make sure the solution is at room temp for 30-60 minutes before use)
 - Add in order given, otherwise SDS will precipitate (Put the SDS and Proteinase K in just before use)

Procedure (good for 60 – 150 ml cell culture samples up to a cell density of 1×10^7 per ml)

1. Before beginning, make sure a water bath is set to 50°C.
2. Add 2ml lysis buffer to 14 ml sarstedt tube (Catalog # 55-538 (tube) 65-816 (cap)) for each sample to be taken.
 - a. This should be done using a baked glass pipette or an RNase free plastic pipette tip.
3. Place tubes for lysis buffer in water bath (50°C) before sampling
 - a. While this is not strictly necessary, it provides the correct conditions for the proteinase-K to be active (inactivates nucleases before they have a chance to degrade the RNA)
4. Remove sample from cell culture and use RNase free 50 ml centrifuge tubes. Pellet the cells by centrifugation at 5,000 rpm for 5 minutes in JA-17 or JA-20 rotor.
5. Keep a beaker next to centrifuge and pour off supernatant immediately (so that you don't lose cells).
6. Resuspend cells to a **final** volume of 2 ml
 - a. **** It is very important that the final volume of the resuspended cell solution is exactly 2 ml – not more! not less! If it is more, then the SDS concentration is lower and RNA can degrade. If it is less, then your salt concentrations are wrong and may affect partitioning of nucleic acids at subsequent extraction steps.****
 - b. I have found the easiest way to resuspend pellet is to:
 - i. Remove the cap of the centrifuge tube and place upside down on lab-bench
 - ii. Pipette 2 ml milli-Q water into cap of centrifuge tube or into a secondary tube or vessel of some kind.

- iii. Add some water (0.5 – 0.75 ml) to cell pellet. Resuspend by either gently shaking tube or pipetting up/down.
 - iv. Using pipette, make sure that the final volume is as close to 2 ml as possible.
 1. Do this by sucking up cell solution carefully, if more water is necessary to make up for the desired volume, suck up more into the pipette tip from the water previously placed in the centrifuge cap (or other secondary tube).
 - v. Pipette resuspended cell solution into warm (50°C) lysis buffer solution.
 - vi. Shake to mix lysis buffer and cell solution thoroughly.
7. Place tubes on rocker for 20 minutes at room temperature.
 8. To freeze samples for processing later, flash freeze at this point in liquid nitrogen and place at -80C until ready for further processing.