From KH 19 Aug. 1990
1 Mar. 2007

Chlamydomonas reinhardtii RNA Prep

2x Lysis Buffer

RNase – Free Stock

1 M Tris-Cl, pH 7.5	2 mL
5 M NaCl	1.2 mL
0.5 M EDTA, pH 8	1.2 mL
H_2O	7.2 mL
10% SDS (Fisher)	8.0 mL*
4 mg/mL Proteinase K	0.4 mL**
(Dec 06: from Fisher Order #24568-2)	20 mL → (Adjust volume for >12 samples)

*Note

- Add in order given, otherwise SDS will precipitate (Put the SDS and Proteinase K in just before use)

**Note

- Make Proteinase K solution (approx. 1 mL) and let stand at room temperature for 30-60 minutes prior to making Lysis Buffer

Procedure

1. Use bottles or tubes that are set aside for RNA work. Harvest cells (60-150 mL) by centrifugation for approx. 2 minutes @ 5,000 rpm (chose rotor based on volume of cells). Cells should make a nice pellet.

2. Pour off supernatant and aspirate with baked Pasteur pipette to avoid losing cells.

3. Resuspend in 1.5 mL H_2O with the Pasteur pipette and transfer cells to baked 30 mL glass beaker OR transfer to 14 mL sarstedt tubes. Use rubber bulb that is set aside for RNA work.

4. Add 1.5 mL 2x Lysis Buffer to cell suspension and stir slowly for 20 minutes with baked stir bar. (Rock on rocker if you use tubes).

5. Set up 14 mL sarstedt tubes for phenol/$CHCl_3$ extractions.

6. Extract cell suspension with 1.5 mL RNase-free phenol. Shake well for one full minute.

7. Add 1.5 mL $CHCl_3$/Isoamyl Alcohol (24:1) and shake well for 1 minute (see #6 above).

8. Centrifuge @ 10,000 rpm for 10 minutes (JA 20 or JA 17) (Use rubber adapters).

9. Collect upper, aqueous phase (avoid interface material) and repeat steps 6-8. (Total of 4 Phenol/ $CHCl_3$ extractions). Do not worry if phase is pink. If you wish, you can add $CHCl_3$ (step 7) at same time. This makes the prep go much faster.

10. Extract twice with $CHCl_3$/Isoamyl Alcohol (you can reduce centrifuge time to 5 minutes each).

11. Remove upper, aqueous phase and precipitate with 2.5 volumes of cold 100% ethanol >= 4 hr @ -20ºC.

12. Collect pellet by centrifugation at 10,000 rpm for 30 minutes (JA 20, JA 17) at 4ºC.

13. Wash pellet in 70% cold ethanol and dry in the speed vac.

14. Resuspend pellet in 0.2 to 0.5 mL H_2O (volume depends on volume and cell density of harvest).

15. Measure OD_{260} and OD_{280} of a 1:50 dilution.

16. mg/mL = OD_{260} X 2.0 (1 OD_{260} = 40 µg/mL RNA)

17. Label and store @ -80ºC (RNA has been quite stable >= 1 year).

Notes

- Yield is generally approx. 1 mg/100 mL @ 5 X 10^6 cells/mL. If you wish to harvest more cells than 150 mL, you should increase H_2O and 2x Lysis Buffer to 3 mL each, and adjust phenol and $CHCl_3$ volumes accordingly.

- This procedure gives excellent RNA, suitable for RNA Blot analysis. A more extensive procedure, utilizing LiCl precipitation of RNA, is given in the lab methods book.