

ISOLATION OF CYTOPLASMIC RNA

Materials:

- (i) Lysis Buffer:
Autoclave these components
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|--------------------------------------|
| 0.1 M NaCl |
| 10 mM Tris pH 8 (lower pH also o.k.) |
| 2 mM EDTA |
| 1% NP-40 (TX-100 also o.k.) |
| 0.5% NaDOC (sodium deoxycholate) |
| 1% β -Mer |

You may find it convenient to make the buffer as needed from concentrated stock solution.

- (ii) Proteinase K (Boehringer Mannheim) 10 mg/ml solution (in H₂O) stored frozen in small aliquots.
- (iii) 3 M NaOAc pH 5.5 [pH anywhere 5-6 o.k. (w/HOAc)]
- (iv) 20% SDS
- (v) Phenol: Chloroform: isoamyl alcohol (25:24:1)
Phenol is saturated w/0.1 M Tris,pH8,
5 mM EDTA; Phenol also contains 0.1% 8-hydroxyquinoline (added as a preservative).
- (vi) Chloroform: Isoamyl alcohol (24:1)

Procedure:

1. Lyse cells in an "appropriate" (at least several volumes) of cold lysis buffer. Suggestion: one plate (100mm) of cells (10^6 - 10^7 cells) can be washed and transferred to an eppendorf tube; lysis is done with 400 μ l of buffer, and then all the manipulations can be performed in an eppendorf tube.
2. Homogenize the cells with any convenient means at your disposal e.g., vigorous vortexing, repeated pipetting with a 1000 μ l pipet tip, passage through a syringe and 25g or 27g needle, Dounce, etc.
3. Spin out the nuclei and debris (if in an eppendorf tube, 3 min in a microfuge (cold) is sufficient. Otherwise, 5000g for 10 min.
4. Transfer the supernatant to a fresh tube. Add SDS to 0.5% and Proteinase K to 250 μ g/ml.
5. Incubate @ 40°-50°C for 30'-60' (Prot. K is stable for up to 60°C).
6. Extract one time with phenol/chloroform/Isaomyl-OH. After centrifuging, the interface should be fairly clean if the Proteinase K worked.
7. Transfer the aqueous (upper) phase to a fresh tube. Extract one time with chloroform/isoamyl-OH. (This step is optional; it seems to get rid of residual phenol).
8. Centrifuge and transfer the aqueous phase to fresh tube. Add NaOAc to 0.15 M(1/20 vol.) and 2.5 vol. cold absolute ethanol. Incubate @ -20°C for 2 hours to overnight or @ -80°C for 20 min.

9. Spin out RNA (e.g., 10-15 min in microfuge) and resuspend in H₂O.

Typical yield: for HeLa or 293 cells, $\sim 10^7$ cells gives ~ 100 μ g of total cytoplasmic RNA.

Comments:

- (i) Most cell types are compatible with this procedure. With others, the high concentration of detergents will lyse the nuclei and give a goopy mess. If this is the case, use milder lysis conditions (e.g., RSB[10mM NaCl, 10mM Tris pH7.4, 3mM MgCl₂] with 0.5% NP-40) for lysis.
- (ii) Frozen cell pellets can be lysed as well, but require vigorous means of homogenization (e.g., passage through a 25g or 27g needle).