PROTOCOL FOR TOTAL RNA ISOLATION USING RNAGENT KIT

Lan Zhou 11/01/00, Commented by TCH

1. Aspirate the medium from the Flask (T-75).
2. Add 3ml ice-cold Denaturing Solution, and let it cover all surface of cells.
3. Shake the flask slowly.
4. Collect the lysate into 15ml- or 50ml-conical tubes; it should be thick and snotty [**Note**: **Cell scrapers may be used to collect the lysate**]. **Keep the lysate on ice**.
5. Add 300ul 2M NaAc (supplied w/ kit), mix well and **keep on ice**.
6. Add 3ml phenol-chloroform (supplied w/ kit), vortex vigorously; then divide into three to four 2ml microfuge tubes.
7. Spin down at top speed for **10 minutes in the cold room**.
8. Transfer the supernatant to a new set of 1.5ml microfuge tubes, and avoid touching the cloudy interphase. [**Optional:** *the supernatant can be pooled and extracted with equal volume of phenol-chloroform. This should further improve the RNA quality*].
9. Add 3-5ul **glycogen** and 1ml (or at least equal volume of the supernatant) isopropanol to each 1.5ml tube; mix well. [**Note: seeDNA cannot be used as a carrier as it interferes with the OD readings at 260nm**].
10. Incubate @ -80^{o}C freezer for 2-4 hours or overnight [the tubes can be stored for a couple of days if you don't use it immediately].
11. Spin down @ top speed for **10 minutes in the cold room**; aspirate the supernatant.
12. Wash pellets with 600ul 70% EtOH, spin down for 5 minutes **in the cold room**; Repeat the wash once. **Whenever possible, try to combine the pellets for one sample into one tube**.
13. Dissolve pellets in 50ul RNase-free water [**Note**: if it is difficult to dissolve, you can incubate the tube briefly at 37C water bath].
14. Check RNA concentrations and quality (**typical yield: 100-200ug per T-75**).