83913 RNA Isolation kit

Product Description:
This RNA isolation kit is based on a single-step extraction method described by Chomczynski and Sacchi[1]. It allows isolation of RNA in about 4h and provides both high yield and purity of undegraded RNA preparations. By eliminating ultracentrifugation, this procedure enables the simultaneous processing of a large number of samples. It is well-suited to both small scale (3 mg tissue or 10^6 cells) and large scale (30 g tissue/RNA) preparations.

Kit Components:
1. Denaturing solution (100 ml)
2. β mercaptoethanol (1ml)
3. 2M sodium acetate solution, pH 4.0 (10ml)
4. Phenol saturated with water (75ml)
5. Chloroform:Isoamylalcohol 49:1 (15ml)
6. Isopropanol

Notes: The denaturating solution, β-mercaptoethanol and phenol should be stored at 4°C. All other components are stable at room temperature. The Kit contains enough reagents to isolate the total RNA from at least 7g of tissue.

Equipment:
Care must be taken to ensure that all equipment (homogeniezer, tubes, etc) be as free from RNases as possible. Tratment with diethyl pyrocarbonate (2) and autoclaving is recommended for all equipment.

RNA Isolation from Animal Tissue
The following protocol describes the isolation of RNA from 1g of tissue.

Extraction:
1. Prepare solution D by adding 100µl of β-mercaptoethanol to 14ml of denaturating solution per gram of tissue. This solution can be stored one month at room temperature.
2. Immediately after removal from the animal (or from liquid nitrogen), mince the tissue on ice and homogenize with 10 ml of solution D in a glass-teflon homogenizer. Transfer to a 50 ml polypropylene tube. Homogenization can be done directly in the 50ml tube with an electric homogenizer.
3. Add 1ml of 2M sodium acetate solution, pH 4.0. Mix thoroughly by inversion.
4. Add 10 ml of water-saturated phenol. Avoid taking from the water phase. Mix thoroughly by inversion.
5. Add 2ml of chloroform:isoamylalcohol mixture 49:1.
6. Shake vigorously for 10s and cool the well-mixed suspension on ice for 15 min.
7. Transfer the sample to a tick-walled pre-cooled 50 ml centrifuge tube and centrifuge at 10´000g for 20 min at 4°C. RNA will be present in the upper aqueous layer whereas DNA and proteins will be present in the interphase and lower phenol phase.

Precipitation:

8. Transfer the aqueous phase to a fresh tube and mix with an equal volume of isopropanol.
9. Precipitate RNA by placing the sample at -20°C for at least 1h.
10. Recover the precipitated RNA by centrifugation at 10´000g for 20 min at 4°C. Remove supernatant and discard.
11. Dissolve the RNA pellet in 3 ml of solution D. Gentle pipetting of the pellet may be required to get some pellets into solution.
12. Repeat precipitation by adding 3 ml of isopropanol, mixing well and chilling at -20°C for 1h.
13. Centrifuge at 10´000g for 10min at 4°C.

Washing:

14. Remove supernatant and wash the RNA pellet in 75% ethanol (pre-cooled at -20°C) for 1h.

Solubilization:

15. Dissolve the RNA in 500µl of 0.5% SDS at 65°C for 10 min or in 1mM EDTA, pH 8.0, or in water. It is recommended to use 0.5% SDS, a weak inhibitor of ribonuclease. For storage, resuspend the RNA in a small volume of water, add 3 volumes of ethanol and store at -70°C.

Note: Be sure to treat the solutions used with diethyl pyrocarbonate (DECP) before resuspending RNA (Tris solutions cannot be DCEP treated).

16. At this point the RNA preparation can be sued for poly $(A)^+$ selction for use in subsequent cDNA synthesis and cloning, Northern blot analysis, dot blot hybridization or translation studies.

RNA Isolation from Tissue Culture Cells Grown in Suspension

The following protocol is for 10^8 cells grown in suspension. The volume of reagents can be varied proportionately for more or less cells.

1. Prepare solution D by adding 100µl of β-mercaptoethanol to 14ml of denaturating solution per 10^8 cells. This solution can be stored one month at room temperature.
2. Add 10ml of solution D per 10^8 cells. Mix thoroughly and allow to sit 1 min at room temperature.
3. Continue with step 3 from RNA isolation from Animal Tissue protocol.

RNA Isolation from Tissue Culture Cells Grown in Suspension

The following protocol can be used for up to 10 culture dishes 10 cm in diameter.

1. Prepare solution D by adding 100µl of β-mercaptoethanol to 14ml of denaturating solution per 10 tissue culture plates. This solution can be stored one month at room temperature.
2. Add 10 ml of solution D to a tissue culture plate. Swirl gently for 30 seconds. Transfer solution D with pipette to the second tissue culture plate and repeat. Continue for up to 10 plates (for single plates, use 1.8 ml of solution D and adjust subsequent reagent volumes accordingly).
3. Continue with step 3 from RNA isolation from Animal Tissue protocol.

References

1. P. Chomczynski, N. Sacchi, Anal. Biochem. 162, 156 (1987)
2. T. Maniatis et al., in Molecular Cloning. A laboratory Manual. P. 7.3, CHS Lab., N.Y. (1989)

Precautions and Disclaimer:

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.