This protocol is for use with samples lysed in 1 ml RNA-Solv Reagent. When processing samples lysed in greater volumes of RNA-Solv Reagent, the volumes of the other reagents and solutions used in the procedures below should be adjusted accordingly.

Isolation of genomic DNA from the organic phase

Complete the RNA preparation before carrying out the procedures below for isolation of genomic DNA from the interphase. If necessary, the interphase and phenol phase can be stored at 4°C overnight.

Procedure

1. Remove any residues of the aqueous phase.
2. Add 0.3 ml of 100% ethanol to the interphase and phenol phase, and carefully mix samples by inversion.
3. Incubate samples at room temperature (15–25°C) for 2–3 min.
4. Centrifuge at 2000 x g for 3-5 min at 4°C to sediment DNA.
5. Remove the phenol/ethanol supernatant and save for subsequent protein isolation.

Store the phenol/ethanol supernatant at 4°C, or start from step 5 in protocol 2 to isolate protein immediately.

6. Add 1 ml 0.1M sodium citrate 10% ethanol to the DNA pellet. Incubate at room temperature for 30 Min, with mixing by inversion every 5 min.
7. Centrifuge at 2000 x g for 5 min at 4°C, and remove the supernatant.
8. Repeat steps 6 and 7 twice.

After this wash step the DNA pellet can be stored in 75% ethanol at 4°C For storage, remove the sodium citrate solution and add 2 ml 75% ethanol without redissolving the pellet.

9. Add 2 ml of 75% ethanol to the DNA pellet. Incubate at room temperature for 20 min, with mixing by inversion periodically.
10. Centrifuge at 2000 x g for 5 min at 4°C and completely remove the ethanol supernatant.
11. Air-dry the DNA pellet for 5–15 min.
12. Redissolve the pellet in 300-600µl of 8 mM NaOH
13. Centrifuge at 12,000 x g for 10 min at room temperature to remove insoluble material,

and transfer the supernatant to a new tube.

The pH of 8 mM NaOH is approximately 9. For storage, the pH of the DNA sample solution should be adjusted to pH 7–8 by addition of TE or HEPES buffer.

14. To neutralize the DNA sample add 60 µl 0.1 M HEPES and 5.5 µl 100 mM EDTA (final concentration 1 mM) per 500 µl 8 mM NaOH used for redissolving the DNA pellet in step 13.

Once the pH is adjusted, DNA can be stored long term at 4°C or –20°C.

Isolation of Protein from the Organic Phase

1. Remove any residues of the aqueous phase.

2. Add 0.3 ml of 100% ethanol to the interphase and phenol phase, and carefully mix samples by inversion.

3. Incubate samples at room temperature (15–25°C) for 2–3 min.

4. Centrifuge at 2000 x g at 4°C for 2 min to sediment DNA.

5. Transfer the phenol/ethanol supernatant containing the protein fraction to a new safe-lock reaction tube.

The DNA pellet can be washed in sodium citrate and stored in 75% ethanol at 4°C for over 3 months (see protocol 1, step 6–8).

6. Add 1.5 ml isopropanol to precipitate the protein, and mix by inversion for 15 s.

7. Incubate samples at room temperature (15–25°C) for 10 min.

8. Centrifuge at 12,000 x g for 10 min at 4°C and remove the supernatant.

9. Add 2 ml guanidine–ethanol solution to the pellet containing the protein, and incubate at room temperature for 20 min.

The protein pellet can be stored in guanidine-ethanol solution at 4°C (for at least 1 month) or –20°C (for at least 1 year).

10. Centrifuge at 7500 x g for 5 min at room temperature, and remove the supernatant.

11. Repeat steps 9 and 10 twice.

12. Add 2 ml of 100% ethanol to the pellet containing the proteins and vortex. Incubate at room temperature for 20 min.

13. Centrifuge at 7500 x g for 5 min at room temperature, and remove the supernatant.

14. Air-dry the pellet for 5–10 min.

Do not dry under centrifugation, as the pellet will be more difficult to dissolve.

15. Add 50 µl urea/DTT solution, and break up the pellet using a needle.

16. Add 450 µl urea/DTT solution, and incubate at room temperature for 1 h.

17. Incubate at 95°C for 3 min then place the tube on ice. During the incubation on ice sonicate 10 times using short bursts.

All proteins should be in solution. If not, repeat step 17 once or twice. Sonication should be performed on ice to avoid foaming of the sample.

18. Centrifuge at 10,000 x g for 10 min at room temperature. Transfer the supernatant containing the proteins to a new tube.

Proteins dissolved in urea/DTT solution can be directly analyzed by SDS-PAGE/Western blot or protein assays such as Bradford, or stored at 4°C overnight or at –20°C for at least 1 year.