

Protocol for RNA Extraction from prostate tissues

Materials

1. Trizol Reagent (Invitrogen)
2. Isopropyl alcohol
3. 75% Ethanol
4. RNase free water
5. 22 Gauge Needle
6. 1 ml syringe

Methods

1. Dissect prostate
2. Fix a small piece of prostate in 4% paraformaldehyde for pathological analysis
3. Immediately add 0.5 ml of Trizol reagent into the remaining prostate
4. Homogenize the tissue
5. Pass the homogenized tissue through a 22 gauge needle 10 times to shear the DNA
6. Add additional 0.5 ml Trizol reagent
7. Incubate samples for 5 minutes at room temperature
8. Add 0.2 ml of Chloroform per 1ml of Trizol reagent
9. Shake tubes vigorously by hand for 15 seconds and incubate at room temperature for 2-3 minutes
10. Centrifuge at 12,000xg for 15 minute at 4°C
11. After centrifugation the Trizol separates into a lower red phenol chloroform phase, an interphase and an upper aqueous clear phase
12. Take the upper phase into a fresh tube
13. Precipitate RNA by adding 0.5ml isopropyl alcohol per 1ml of Trizol reagent
14. Incubate samples for 15 minutes at room temperature
15. Centrifuge at 12,000xg for 10 minutes at 4°C
(may be able to see RNA pellet after centrifugation)
16. Remove supernatant
17. Add 1.0 ml 75% ethanol to wash the pellet (RNA)
18. Vortex it for 15 seconds
19. Centrifuge at 12,000xg for 10 minute at 4°C
20. Dry the pellet at room temperature
21. Re-suspend the pellet in 10µl RNase free water
22. Quantitate RNA by spectrophotometer at 260 nm
23. Store RNA at -20°C
24. Run on a gel to check the quality