University of Al-Maarif

Medical Laboratory Technique Department.

2nd. Stage

Practical Molecular Biology

RNA extraction by TRIzol™

1. Collect of Blood in EDTA Tube from patient and control

2- Tow hundred of EDTA blood suspend it with trizol 0.6 ml

3. Fifteen minutes incubation to permit complete dissociation of the nucleoproteins complex .

4. The chloroform was added as 200µl of TRIzol™ Reagent used for lysis .

5. Incubation for 2–3 minutes at room temperature.

6. The sample was centrifuged for 10 minutes at 10,000 rpm. The mixture was separated into a lower red phenol-chloroform, interphase, and a colorless upper aqueous phase.

7. The aqueous phase containing the RNA transferred to a new tube .

8. The RNA was precipitated by adding 200 µl of isopropanol or absolute ethanol to the aqueousphase.

9. The mixture incubated for 2minutes at room temperature

10. The mixture was centrifuged for 10 minutes at 10,000 rpm. Total RNA precipitate on filter of spin column tube.

11. The supernatant was discarded.

12. The column gets re-suspended by 0.5 ml of washing buffer 1.

13. The tube was centrifuged for 2 minutes at 10000rpm.

14. The supernatant was discarded.

15. The column gets re-suspended by 0.5 ml of washing buffer 2.

16. The tube was centrifuged for 2 minutes at 10000rpm.

17. The supernatant was discarded.

18. The column gets pre-heated elution by 75 µl and centrifuged 1 min at 10000rpm .

17 .Total RNA samples stored in deep freezer.