

RNA isolation using TRIzol reagent

I. Homogenization

1. Prepare TRIzol reagent in a 50 ml Screw Cap tube at room temperature (RT) before taking the frozen specimen out as described in the table.

Tissue (mg)	TRIzol (ml)	TRIzol (ml) for difficult tissue (liver, spleen, bone)
50- 100	1	2
500	5	10
1000	10	20

Sample volumes should not exceed 10% of the volume of TRIzol reagent used for homogenization.

2. As soon as the frozen tumor specimen is removed from the freezer, cut it off into small pieces (approximately 50-100 mg) while it is still frozen.

3. Immediately place into TRIzol reagent in 50 ml Screw Cap tube, and homogenize using a PowerGen 125 Tissue Homogenizer for 30- 60 seconds (starting at 5000 rpm and going up to 20,000 rpm).

4. Incubate at RT (Room Temperature) for 5-10 minutes after homogenization.

II. Phase separation.

5. Transfer the homogenate to 50 ml Oak Ridge Centrifuge tube and centrifuge at 12,000 g for 5-10 minutes at 4°C. The resulting pellet contains extra-cellular membranes, polysaccharides and high molecular weight DNA while the supernatant contains RNA.

6. Remove the upper fat layer by using a Pasteur pipette hooked up to a vacuum flask. Transfer supernatant to a new Oak Ridge Centrifuge tube.

7. Add 0.2 ml Chloroform per 1 ml TRIzol reagent used. Shake tube vigorously for 15- 30 seconds by hand and incubate at RT for 5-15 minutes.

8. Centrifuge at 12,000 g for 15 minutes at 4°C. Carefully remove the upper aqueous phase which contains the total RNA, and place this in a new 50 ml Oak Ridge centrifuge tube.

9. Preserve bottom layer at 4°C for subsequent isolation of DNA and proteins.

III. RNA precipitation and wash.

10. Add 0.5 ml Isopropyl alcohol per 1 ml of TRIzol reagent used and incubate RT 10 min. Centrifuge 12,000 g for 8 min at 4°C.

11. Wash the pellet with at least 1 ml of 75% ETOH per 1 ml TRIzol used, centrifuge 12,000 g for 5 min at 4°C. Wash one more time with 75% ETOH.

12. Dry the pellet at RT (do not dry completely) and re-suspend the RNA pellet in 200 –300 µl DEPC –TE.