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.

<table>
<thead>
<tr>
<th>Tissue (mg)</th>
<th>TRIzol (ml)</th>
<th>TRIzol (ml) for difficult tissue (liver, spleen, bone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50–100</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>500</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

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.