

Processing of Microdissected Tissue for Molecular Analysis RNA-based Studies

[Total RNA Isolation](#)

[Optional DNase Treatment](#)

This method was successful in our lab using prostate tissue and for our specific objectives. Investigators must be aware that they will need to tailor the following protocol for their own research objectives and tissue under study.

Total RNA Isolation

Recovery and analysis of RNA from frozen tissue sections can be achieved using slight modifications of standard methods. We obtain total RNA from microdissected samples using the Micro RNA isolation kit (Stratagene, La Jolla, CA).

Materials:

1. Stratagene Microisolation Kit.
2. Before beginning, clean all pipettors with RNase away or a similar product.

Method:

1. Place the LCM cap on an Eppendorf tube containing 200 μ l RNA denaturing buffer (GITC) and 1.6 μ l b-mercaptoethanol. Invert several times over the course of 2 minutes to digest the tissue off the cap.
2. Remove the solution from the reagent tube and replace it in a sturdy RNase free 1.5 ml tube.
3. Add 20 μ l (0.1X volume) 2M sodium acetate, pH 4.0.
4. Add 220 μ l (1X volume) water saturated phenol (bottom layer; 1:1).
5. Add 60 μ l (0.3X volume) chloroform-isoamyl alcohol.
6. Shake the tube vigorously for 15 seconds.
7. Place on wet ice for 15 minutes.
8. Centrifuge for 30 minutes at 4°C to separate the aqueous and organic phases.
9. Transfer upper aqueous layer to a new tube.

TIP: If any of the lower organic phase was accidentally transferred and may be contaminating the aqueous phase, this will interfere with the subsequent isopropanol precipitation. To remove any residual organics from the aqueous layer, add one volume of 100% chloroform, mix well, and centrifuge for 10 minutes at 4°C to separate the aqueous and organic phases. Transfer the upper layer to a new tube.

10. Add to aqueous layer, 1-2 μ l glycogen (10 mg/ml) and 200 - 300 μ l cold isopropanol (i.e., equal volume).

TIP: Glycogen facilitates visualization of the pellet, which can be problematic when using small amounts of RNA.

11. Place samples at -80°C for at least 30 minutes. It may be left overnight.
TIP: Before centrifuging, the tubes may need to be thawed slightly if they have solidified during the isopropanol precipitation.
12. Centrifuge for 30 minutes at 4°C with cap hinges pointing outward so that the location of the pellet can be better predicted.
13. Remove the supernatant and wash with 300 μl cold 70% ethanol. Add the alcohol and spin for 5 minutes at 4°C .
14. Remove the supernatant.
15. Let the pellet air dry on ice to remove any residual ethanol. Overdrying prevents the pellet from resuspending easily.
16. The pellet may be stored at -80°C until use or proceed to DNase treatment (below).

Optional DNase Treatment

DNase treatment is highly recommended for microdissected cells. Genomic DNA contamination is often problematic with these samples, possibly due to the small DNA fragments that are created during tissue processing and are difficult to purify from RNA.

1. To RNA pellet, add 15 μl DEPC water and 1 μl 20 U/ μl RNase inhibitor (Perkin Elmer).
2. Gently mix by flicking until the pellet is dissolved.
3. Quickspin.
4. Add 2 μl 10X DNase buffer (GenHunter) and 2 μl 10 U/ μl DNase I (GenHunter; 20 units total)
5. Incubate at 37°C for 2 hours.
6. Re-extract RNA by adding:
 - 2 μl 2M sodium acetate, pH 4.0
 - 22 μl water saturated phenol
 - 6 μl Chloroform-isoamyl alcohol
7. Vortex vigorously for 15 seconds.
8. Place on wet ice for 15 minutes.
9. Centrifuge 10 minutes at 4°C .
10. Transfer upper layer to a new tube.
TIP: If any of the lower organic phase was accidentally transferred and may be contaminating the aqueous phase, this will interfere with the subsequent isopropanol precipitation. To remove any residual organics from the aqueous layer, add one volume of 100% chloroform, mix well, and centrifuge for 10 minutes at 4°C to separate the aqueous and organic phases. Transfer the upper layer to a new tube.
11. Continue with RNA extraction from Step 10 in Total RNA Isolation, adjusting the volume of isopropanol accordingly.