

Small scale extraction of total RNA from *Drosophila melanogaster*

Overview

The RNA that is to be labelled must be of high quality. It must be undegraded and contain no genomic DNA contamination. Several extraction methods have been tested for use with *Drosophila* samples. However, extraction using TRIzol gives consistent, reliable results and is considerably cheaper than kit-based products and is therefore our method of choice.

Poly A+ mRNA constitutes approximately 2% of total RNA from a *Drosophila* embryo. Labelling of 50 µg total RNA using an oligo(dT) primer gives similar results to approximately 1 µg poly A+ RNA and it is therefore unnecessary to purify poly A+ RNA from the total RNA prep.

This protocol is based on a method from Kevin White's web site (<http://quantgen.med.yale.edu/>). This protocol has been optimised to extract 1 to 10 µg total RNA. Please ensure that you have a sufficient amount of tissue before sending us your samples ([recommended tissue amounts](#)).

Equipment and reagents

- TRIzol (Gibco/BRL; Cat. No. 15596-018)
- DEPC - Diethyl pyrocarbonate (BDH; Cat. No. 44170 3D)
- 1.5ml disposable Polypropylene Pellet Pestle with microtube (Anachem; Cat. No. K-749520-0000). Autoclave in DEPC-treated water to ensure that RNase-free
- Chloroform, (BDH; Cat. No. 100775A)
- Isopropanol (BDH; Cat. No. 102246L)
- DEPC-treated MilliQ water
- 70% ethanol/DEPC MilliQ water
- RNAlater (Ambion; Cat. No. 7020)
- Micro 20 centrifuge, Hettich
- GeneElute Linear Polyacrylamide (Sigma; Cat. No. 5-5675)

Removal of RNase

All materials should be autoclaved and only handled using gloves. Glassware should be baked at 180 °C overnight. Water and solutions should be treated with DEPC. The work area can be cleaned using RNase Zap to further limit the risk of RNase contamination. If at all possible, it is also a good precaution to use a separate set of pipettes for RNA work.

Procedure

1. For adult flies, imaginal discs and other tissues, transfer tissue to a 1.5 ml microfuge tube. For embryos, dechorionate first, rinse thoroughly with water and blot off excess before weighing (do not fix!). If samples are ready to be homogenised immediately, skip to step 2. If samples are not yet ready for processing, then either:
 - ◆ flash freeze tube in liquid nitrogen then store in -80 °C freezer until ready to homogenise. Thaw on ice before continuing with step 2, or;
 - ◆ Add 5 volumes of RNAlater. The tissue can be stored safely at 25 °C for a couple of days, at 4 °C for up to a week, and at -20 °C or -80 °C for at least a month. When ready to continue, remove RNAlater before continuing with step 2.

2. Place sample on ice and add 300 μ l TRIzol.
3. Homogenise using an RNAase-free polypropylene pellet pestle. Avoid making sample hot.
4. At this point the sample can be stored at -80 $^{\circ}$ C until ready to be sent to us on dry ice.
5. Thaw sample on ice. Add 0.2 μ l GeneElute Linear Polyacrylamide (25 μ g / μ l)
6. Centrifuge at 13,000 rpm in a microcentrifuge for 10 minutes to pellet debris such as the chorion, vitelline membrane, cuticle etc.
7. Transfer supernatant to a fresh 1.5 ml tube.
8. Add 0.2 volumes chloroform, vortex for 60 seconds.
9. Centrifuge at 13,000 rpm for 15 minutes.
10. Remove upper phase to a new RNase-free tube, being careful not to touch the interface.
11. Add 0.8 volumes of isopropanol, invert and then incubate for 1 hour at -20 $^{\circ}$ C.
12. Pellet the RNA by centrifugation at 13,000 rpm for 15 minutes.
13. Discard the supernatant and wash the RNA pellet with 500 μ l 70% ethanol/DEPC MilliQ water.
14. Air dry the pellet briefly (leave on work bench). Resuspend in an appropriate volume of DEPC MilliQ water, e.g. 5 μ l. The RNA will dissolve more readily if the DEPC MilliQ water is preheated to 55 $^{\circ}$ C.
15. Verify the quality of RNA by gel electrophoresis of 0.5 μ l. Stain the gel using SYBR Gold instead of Ethidium Bromide. Do not quality control the RNA using the Nanodrop, if you do there won't be enough total RNA for the amplification.
16. The remaining 4.5 μ l of total RNA can now be used for amplification.

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