

Standard protocol for the 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/>). We only require 50 µg total RNA per labelling reaction and this protocol has been optimised to extract this amount of 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
- RNeasy (Qiagen; Cat. No. 7020)
- Micro 20 centrifuge, Hettich

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 and weigh on microbalance. 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 RNeasy. 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 RNeasy before continuing with step 2.

2. Place sample on ice and add 300 μ l TRIzol.
3. Homogenise the sample for 30-60 seconds using a disposable polypropylene pellet pestle and microtube. Avoid making sample hot.
4. At this point the sample can be stored at -80 °C until ready to be sent to us on dry ice.
5. Thaw sample on ice. Depending on the amount of tissue add up to 700 μ l of TRIzol. Centrifuge at 13,000 rpm in a microcentrifuge for 10 minutes at 4 °C to pellet debris such as the chorion, vitelline membrane, cuticle etc. Transfer supernatant to a fresh 1.5 ml tube.
6. Add 0.2 volumes chloroform, shake vigorously for 15 seconds and incubate at room temperature for 2-3 minutes.
7. Centrifuge at 13,000 rpm for 15 minutes at 4 °C.
8. Remove upper phase to a new RNase-free tube, being careful not to touch the interface. Discard tube with lower phase and interface.
9. Add 0.7 volumes of isopropanol to precipitate the RNA. Incubate at room temperature for 5 minutes or 1 hour at -20 °C and then centrifuge at 13,000 rpm for 15 minutes at 4 °C.
10. Discard the supernatant and wash the RNA pellet with 1 ml 70% ethanol/DEPC MilliQ water. Centrifuge at 13,000 rpm for 10 minute at 4 °C.
11. Air dry the pellet briefly (leave on work bench). Resuspend in an appropriate volume of DEPC MilliQ water, e.g. 20 to 50 μ l. The RNA will dissolve more readily if the DEPC MilliQ water is preheated to 55 °C.
12. Verify quality of RNA according to the RNA quality control / assessment protocol.

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