RNA Extraction from Drosophila Tissues using TRIzol Reagent Version 2

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Abstract

This protocol is adapted from the Invitrogen Life Technologies Trizol manual.

Citation: Elizabeth Allen RNA Extraction from Drosophila Tissues using TRIzol Reagent. **protocols.io** dx.doi.org/10.17504/protocols.io.fgtbjwn
Published: 03 Aug 2016

Before start

1. Prepare an **RNase-free** working area, wipe down barrels of micropipettes, use **filter tips** and RNase-free microcentrifuge tubes, and always wear **gloves**.
2. **Snap-freeze tissue** as it is collected by adding tissue to a microfuge tube on dry ice.
3. This protocol calls for **50-100 mg of tissue** to be homogenized in a microcentrifuge tube in **1 mL of TRIzol Reagent**. *Use caution here - homogenization with a motorized homogenizer may result in overflow.

Materials

- Isopropanol by Contributed by users
- Chloroform by Contributed by users

  TRIzol Reagent 15596026 by Thermo Fisher Scientific

  Bio Plas Disposable Homogenization Pestles BPI-4040-PB by Capitol Scientific
- Microcentrifuge Tubes by Contributed by users
- Temperature-regulated centrifuge by Contributed by users
- Ultrapure Distilled, Nuclease Free Water by Contributed by users
- Filter Tips by Contributed by users
- Dry Ice by Contributed by users
- 75% Ethanol by Contributed by users

Protocol

Step 1.

Add 1 mL TRIzol Reagent to 50-100 mg of frozen *Drosophila* tissue in a 1.5 mL microcentrifuge tube, and homogenize immediately with a disposable plastic pestle.

AMOUNT
1 ml Additional info:

REAGENTS
TRIzol Reagent [15596026](#) by [Thermo Fisher Scientific](#)

Step 2.

Centifuge the sample at 12,000 x *g* for 10 minutes at 4˚C.

*Pellet contains ECM, polysaccharides, and high molecular weight DNA; **supernatant contains the RNA**. In high fat samples, a layer of fat collects above the supernatant.

DURATION
00:10:00

Step 3.

Remove and discard the fatty layer.

Step 4.

Transfer the cleared supernatant to a new tube.

Step 5.

Incubate the sample for 5 minutes at room temperature.

DURATION
00:05:00

Step 6.

Add 0.2 mL of chloroform, and cap the tube securely.

REAGENTS
Chloroform by Contributed by users

Step 7.

Shake the tube vigorously **by hand** for **15 seconds**.

DURATION
00:00:15

Step 8.

Incubate for 2-3 minutes at room temperature.

DURATION
00:03:00

Step 9.

Centrifuge the sample at 12,000 x *g* for **15** minutes at **4˚C**.

*The mixture separates into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. **RNA remains in colorless aqueous phase** (50% of the total volume).

DURATION
00:15:00

Step 10.

Remove the aqueous phase of the sample by angling the tube at 45˚ and pipetting the solution out.

Place the aqueous phase into a **new tube**.

*Avoid drawing any of the interphase or organic layer into the pipette.

*The interphase and organic phenol-chloroform phases can be saved for DNA or protein isolation if desired (saved overnight at 4˚C); however, the protocols for these procedures will not be discussed here. Please refer to the manufacturer's TRIzol Reagent manual.

REAGENTS
- Microcentrifuge Tubes by Contributed by users

Step 11.

Add **0.5 mL** of 100% isopropanol to the aqueous phase.

REAGENTS
- Isopropanol by Contributed by users

Step 12.

Incubate sample at room temperature for 10 minutes.

DURATION
00:10:00

Step 13.

Centrifuge at 12,000 x *g* for **10 minutes** at 4˚C.

*RNA is often visible prior to centrifugation, and forms a gel-like pellet on the side and bottom of tube.

DURATION
00:10:00

Step 14.

Remove all supernatant from the tube, leaving the RNA pellet.

Step 15.

Wash the pellet with **1 mL** 75% ethanol.

*RNA can be stored in 75% ethanol at least 1 year at -20˚C, or at least 1 week at 4˚C.

AMOUNT
1 ml Additional info:

REAGENTS
75% Ethanol by Contributed by users

Step 16.

1. Briefly **vortex** the sample.
2. **Centrifuge** the tube at 7,500 x *g* for 5 minutes at 4˚C.
3. Discard the wash.

DURATION
00:05:00

Step 17.

Vacuum or air **dry** the **RNA pelle**t for 5-10 minutes.

*Do not dry the pellet by vacuum centrifuge.

*Do not allow the RNA to dry completely.

DURATION
00:10:00

Step 18.

Resuspend the RNA pellet in RNase-free water by passing the solution up and down several times through a pipette tip.

Step 19.

Incubate in a water bath or heat block set at 55-60˚C for **10-15 minutes**.

DURATION
00:15:00

Step 20.

Proceed to downstream applications, such as DNase treatment or cDNA synthesis, or store at -70˚C

Warnings

TRIzol Reagent and Chloroform are toxic (inhalation, contact, and ingestion). Always use a fume hood, and wear protective clothing, eyeware, and gloves.