Aug 03, 2016 Version 1

# RNA Extraction from Drosophila Tissues using TRIzol Reagent V.1

DOI
dx.doi.org/10.17504/protocols.io.fgrbjv6

Elizabeth Allen[1]

[1]University of Massachusetts Medical School

Elizabeth Allen
University of Massachusetts Medical School





**DOI:** dx.doi.org/10.17504/protocols.io.fgrbjv6

**Protocol Citation:** Elizabeth Allen 2016. RNA Extraction from Drosophila Tissues using TRIzol Reagent. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.fgrbjv6**



**Protocol status:** Working

**Created:** August 03, 2016

**Last Modified:** March 20, 2018

**Protocol Integer ID:** 3313

**Keywords:** rna extraction from drosophila tissue, rna extraction, using trizol reagent, trizol reagent, invitrogen life technologies trizol manual, trizol reagent this protocol, drosophila tissue, rna, trizol, drosophila

## Abstract

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

## Materials

MATERIALS

- Isopropanol
- Chloroform
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Bio Plas Disposable Homogenization Pestles **Capitol Scientific Catalog #**BPI-4040-PB
- Microcentrifuge Tubes
- Temperature-regulated centrifuge
- Ultrapure Distilled, Nuclease Free Water
- Filter Tips
- Dry Ice
- 75% Ethanol

STEP MATERIALS

- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Chloroform
- Microcentrifuge Tubes
- Isopropanol
- 75% Ethanol
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Chloroform
- Microcentrifuge Tubes
- Isopropanol
- 75% Ethanol

## Protocol materials

- Chloroform
- Microcentrifuge Tubes
- Isopropanol
- 75% Ethanol
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Dry Ice
- Isopropanol
- Chloroform
- Bio Plas Disposable Homogenization Pestles **Capitol Scientific Catalog #**BPI-4040-PB
- Filter Tips
- Isopropanol
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Chloroform
- Microcentrifuge Tubes
- 75% Ethanol
- Microcentrifuge Tubes
- Temperature-regulated centrifuge
- Ultrapure Distilled, Nuclease Free Water
- 75% Ethanol
- TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026
- Chloroform
- Microcentrifuge Tubes
- Isopropanol
- 75% Ethanol

## Safety warnings

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

## 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.

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.

1 mL

TRIzol Reagent **Thermo Fisher Scientific Catalog #**15596026

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.

00:10:00

3 Remove and discard the fatty layer.

4 Transfer the cleared supernatant to a new tube.

5 Incubate the sample for 5 minutes at room temperature.

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

Chloroform

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

00:00:15

8 **Incubate** for 2-3 minutes at room temperature.

00:03:00

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).

00:15:00

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.

Microcentrifuge Tubes

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

Isopropanol

12 Incubate sample at room temperature for 10 minutes.

00:10:00

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.

00:10:00

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

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.

1 mL

75% Ethanol

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.

00:05:00

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.

00:10:00

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

19 **Incubate** in a water bath or heat block set at 55-60˚C for **10-15 minutes**.
00:15:00

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