# RNA Extraction and Purification with TRIzol® reagent

## Overview

This method is for RNA extraction and purification using the TRIzol® reagent protocol. It includes a step with DNase treatment if pure RNA needs to be isolated. You should plan to spend about 3hrs for a full protocol run.

**RNA is highly unstable at room temperature**, so make sure to work quickly at the start (think minutes) and to preserve samples in -80°C freezer after completion. Tissue preservation prior to RNA extraction is important - tissues should be stored at the -80°C freezer without ethanol, or in RNAlater® reagent.

## Getting Started

This protocol uses chloroform, which produces dangerous fumes, and therefore, the start of this protocol **should be carried out inside the fume hood.** If you include the DNase step, make sure to prepare the reagent **before** starting extraction or **during** the first centrifuge period during **phase separation**. Make sure to use RNase-free supplies, sterile centrifuge tubes, and follow necessary aseptic techniques.

## Preparation of PureLink® DNase Optional Treatment

This is required if you include the optional step to remove DNA contamination from samples. It can also be done after completing RNA extractions, but is more laborious and costly. The kit is located in the 4°C walk-in fridge next to the fume hood (white and red box).

For each reaction, prepare the following mixture for a total of 80 µL to add to each reaction.

| Component | 1x Reaction volume (µL) |
|---|---|
| 10X DNase I Reaction Buffer | 8 |
| Resuspended DNase (~3 U/µL) | 10 |
| RNase free water | 62 |
| Final volume | 80 |

## Preparation of Polytron Tissue Homogenizer*

Before you start homogenizing (or grinding) the tissues samples, you must clean the homogenizer. This will ensure that the homogenizer is sterile and will prevent contamination of your samples. All chemicals should be prepared in sterile 15 mL plastic falcon tubes, clearly labeled and dated. If these chemicals look opaque or dirty, discard them and refill new, clean falcon tubes to 10 mL.

1) Dip the homogenizer tip on **NaOH (1N)** and turn it on to a speed setting of 6 for about 15 to 30 seconds. Turn homogenizer off before removing from vial.

2) Dip the homogenizer tip on **70% Ethanol** and turn it on to a speed setting of 6 for about 15 to 30 seconds. Turn homogenizer off before removing from vial. (Make sure this is not the same ethanol source for following phase separation steps)

3) Dip the homogenizer tip on "**Water 1" (RNase-free water)** and turn it on to a speed setting of 6 for about 15 to 30 seconds. Turn homogenizer off before removing from vial.

4) Dip the homogenizer tip on "**Water 2" (RNase-free water)** and turn it on to a speed setting of 6 for about 15 to 30 seconds. Turn homogenizer off before removing from vial.

*Alternative modes of tissue homogenization include 1) pulverizing samples in liquid nitrogen, 2) grinding with a pestle while immersed in TRIZOL, or 3) bead-beating samples with sterile beads while immersed in TRIZOL.

## Tissue Lysis

These steps are to be carried out **inside the fume hood.**

1) Transfer **1 ml (or 1,000 µL) of TRIzol® reagent** into an RNase-free, sterile 2ml deep centrifuge tubes or vials. Clearly label each tube or vials.

2) Transfer each **frozen tissue sample** to individual centrifuge tube containing TRIzol®. Do not let the tissue sit at room temperature for more than a few minutes, as RNA will degrade quickly as tissues thaw. **If samples are preserved in RNAlater**, take off excess of RNAlater before placing inside tubes with TRIzol® **but do not let samples dry** by air or by other means.

3) Grind or blend samples by dipping the **Polytron Tissue Homogenizer** tip on samples. Start at a low speed (e.g., 6) and increase if needed to 10. Make sure to homogenize samples as much as you can by carefully moving the tube up and down. Use caution as TRIzol® can be splattered during this process. Turn homogenizer off before removing from vial.

4) **Clean homogenizer between samples** by dipping the tip on Water 1 (RNase-free water) and then on Water 2 (RNase-free water) for 15 to 30 seconds (each) at speed 6 or higher.

5) Incubate samples at room temperature for 5 min.

## Phase Separation

All **discarded flow-through** will go in the waste bin **inside the fume hood**.

6) **Inside the fume hood**, Add 200 µL of chloroform (or 50 µL of 4-Bromoanisole) to each sample. Vigorously mix by sealing tubes and inverting it multiple times for ca. 15 seconds, do not vortex.

7) Incubate at room temperature for 2 – 3 minutes.

8) Centrifuge samples for 15 minutes at 12,000 x g, at 4°C (centrifuge inside the cold room).

9) If samples phases aren't clearly separated (there should be a clear, transparent upper phase), repeat **Step 4**.

10) Transfer about **600 µL of the transparent upper phase** (this contains the RNA) to a RNase free centrifuge tube. Carefully do so on 2 – 3 intervals of 200 µL each, to avoid mixing phases. Change pipet tips between samples.

5) Add equal amount of **70% Ethanol** to samples and vortex to mix well. Invert the tube to disperse any precipitate formed by the ethanol. (Make sure this is not the same ethanol used to clean the homogenizer)

## Binding, Washing and Elution

All **discarded flow-through** will go in the liquid waste bin **inside the fume hood**.

11) Transfer up to 700 µL of the mixture to a Spin Cartridge within a 2 mL Collection Tube.

12) Centrifuge at 12,000 x g for 15 seconds at room temperature. Discard the flowthrough and reinsert Spin Cartridge on Collection Tube.

13) Repeat Steps 12 and 13 until all of the sample has been processed.

14) If **NOT** following PureLink® DNase Optional Treatment protocol, add 700 µL of **Wash Buffer I** to the Spin Cartridge and centrifuge at 12,000 g for 15 seconds at room temperature, discard flow through and Collection Tube. Insert the Spin Cartridge on a new collection tube, and skip to **Step 19**. If following the PureLink® DNase Optional Treatment protocol, skip to the next step.

15) If **FOLLOWING** PureLink® DNase Optional Treatment protocol: Add 350 µL of **Wash Buffer I** to the Spin Cartridge and centrifuge at 12,000 g for 15 seconds at room temperature, discard flow through and Collection Tube. Insert the Spin Cartridge on a new collection tube.

16) Add 80 µL of the **PureLink® DNase mixture** directly to the surface of the Spin Colum membrane. Incubate at room temperature for **15 minutes.**

17) Add 350 µL of **Wash Buffer I** to the Spin Cartridge and centrifuge at 12,000 g for 15 seconds at room temperature, discard flow through and Collection Tube. Insert the Spin Cartridge on a new collection tube.

18) Add 500 μL of **Wash Buffer II with Ethanol** to the Spin Cartridge and centrifuge at 12,000 g for 15 seconds at room temperature, discard flow through and reinsert Spin Column to Collection Tube.

19) Repeat **Step 19 once**.

20) Centrifuge Spin Cartridge and Collection Tube at 12,000 g for 1 minute at room temperature to dry the membrane. Discard Collection Tube and place Spin Cartridge on a recovery, RNase-free 1.5 mL centrifuge tube.

21) Elute RNA by adding 30 – 100 μL of **RNase-free water** to the center of the Spin Cartridge. **Incubate** at room temperature for **1 minute**.

22) Centrifuge the Spin Cartridge with the recovery centrifuge tube at ≥ 12,000 g for 2 minutes at room temperature.

23) Repeat **Steps 22 to 23** one to two additional times, for a maximum total of three elution steps.

24) Discard the Spin Cartridge. The recovery centrifuge tube has the extracted RNA. Store extracted RNA samples at -80°C or proceed with other lab protocols right away.