

mi- Total RNA Isolation Kit

Cat. No mi-CZ100

This kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only.

don't risk your experiment. trust ... **metabion**

Introduction

The mi-Total RNA Isolation kit is a ready-to-use reagent for the isolation of total RNA from cells and tissues in animal, plant or bacteria. The operation of the kit is very simple and easy.

First the sample is added into the mi-Total Reagent for complete lysis. Secondly chloroform is added into the solution and then the sample is mixed. Then the mixture will form three phases: upper aqueous phase, middle phase and lower organic phase. The RNA remains exclusively in the aqueous phase. The formed middle phase is tightly condensed so that it is easy for the operator to extract the upper clear phase to improve the purity of the RNA. After transfer of the aqueous phase, the RNA is recovered by the precipitation with isopropyl alcohol.

The total RNA isolated by mi-Total Reagent is free of protein and DNA contamination. It can be used for Northern Blot analysis, Dot Blot hybridization, poly (A)⁺ selection, in vitro translation, RNase protection assay, RT-PCR assay, cDNA library building and other research involving RNA. The mi-Total Reagent facilitates the isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from rat liver denatured by formaldehyde, electrophoresed on an agarose gel, shows three predominated ribosomal RNA bands at 5kb (28S), 2kb (18S) and 0.3kb (tRNA, 5S)

Kit Contents

1 Bottle

mi-Total RNA Isolation Kit

store at 4°C

Required Equipment

Microcentrifuge (13,000 rpm or 12,000 x g)

Microcentrifuge tubes

Distilled water (pH 7-8) or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

Liquid nitrogen (for tissue extraction)

Chloroform

Isopropyl alcohol

75% ethanol (diluted in DEPC-treated water)

RNase-free water (Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave)

(If highly purified RNA is required: saturated phenol (pH 4.5))

(If small RNA amounts are isolated: glycogen)

Kit Storage

Store at 2-8°C and the effective time is one year.

Precautions

See MSDS on our homepage (www.mymetabion.com).

Warning! **This kit is toxic!** In case of contact with skin or eyes, please flush immediately with water. Wear gloves and goggles!

Protocol

Note: Before starting, please read the following...

How to prevent RNase contamination:

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA:

1. Always wear gloves when working with RNA.
2. Sterile, disposable plasticware can safely be considered RNase-free and should be used when possible.
3. Contaminating RNases can be inactivated by baking glassware at 180°C or higher for several hours.
Alternatively, glassware can be soaked in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, drained and autoclaved (necessary to destroy any unreacted DEPC which can otherwise react with other proteins and RNA). DEPC will destroy polycarbonate or polystyrene materials (e.g. electrophoresis tanks), which should instead be decontaminated by soaking in 3% hydrogen peroxide for 10 minutes. Remove peroxide by extensively rinsing with RNase-free water (see below) prior to use.
4. Metal spatulas can quickly be decontaminated by holding in a burner flame for several seconds.
5. It is a good idea to maintain a separate area for RNA work that has its own set of pipettors. This is especially important if your work requires the use of RNase A (e.g. plasmid preps).
6. All steps of the RNA isolation should be performed at 4°C / -20°C on ice !!!

Isolation for special starting materials

1. Extraction of RNA from tissue containing fat such as muscle and the stem in plant, the tissue needs to be separated in by an additional step. The homogenized sample needs to be centrifuged at 13,000 rpm (12,000 x g) for 15 min at 4°C. Then the fat will float on upper and needs to be discarded. RNA will be left in the second phase.
In every case, transfer the clear homogenized solution into a clean tube and add chloroform.
Then perform the following steps described in the protocol (Phase separation).

2. Extraction of RNA from small quantities of tissue (1 to 10 mg) or cell (10^2 to 10^4) samples: add only 800 μ l of mi-Total Reagent to the tissue or cells. Following the sample lysis, add chloroform and proceed with the phase separation as described before. Prior to precipitating the RNA with isopropyl alcohol, add 5-10 μ g of RNase-free glycogen and pass twice through a 6G needle prior to chloroform addition.

RNA Isolation Protocol

Note: All steps should be performed at 4°C / -20°C on ice !!!

Suggested sample volume with 1 ml of mi-Total Reagent for the extraction:

Sample	Maximum volume	Best volume
Tissues from animal / plant	150 mg	50-80 mg
Bacteria	1.5×10^9	1×10^7 - 5×10^8
Yeast	1.5×10^7	5×10^6 - 1×10^7
Cultivated animal cells	1.5×10^7	5×10^6 - 1×10^7

1. Homogenization

- Tissues: Smash the tissue into powder under the liquid nitrogen (N₂). Homogenize the tissue samples with mi-Total Reagent according to the volume recommended before (table). The sample volume should not exceed 10% of the volume of mi-Total Reagent used for homogenization (<100 μ l).
- Cells grown in Monolayer: Lyse the cells directly in a culture dish by adding 1 ml of mi-Total Reagent to a 3.5 cm diameter dish, laying for 3-5 min, shaking 2-3 times in order to lyse cells completely, and transfer the cell lysate into a centrifugal tube. Normally add 1ml mi-Total Reagent for every 20-30 cm² solution (5×10^6 - 1×10^7 cells).
- Cells grown in Suspension: Pellet the cells by centrifugation. Lyse the cells in mi-Total Reagent by repetitive pipetting. Add the sample according to the volume recommended before (table). Washing cells before the addition of mi-Total Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

Please note the amount of added mi-Total Reagent. The addition of the appropriate amount of chloroform, isopropyl alcohol and ethanol in the following steps depends on this amount!!!

After this step, the samples can be stored at -70°C for at least one month.

2. Phase Separation

Incubate the homogenized samples for 5-10 min to permit the complete lysis of the sample.

Add chloroform according to the rate of 1:5 into the mi-Total Reagent solution. (Example: add 200 µl of chloroform to 1000 µl of homogenization mix).

Close sample tubes securely!

Shake the tubes vigorously by hand and incubate them for 2 -3 min.

Spin the samples at 13,000 rpm (12,000 x g) for 15 min at 4°C.

Following centrifugation, the mixture separates into a lower phase, a middle phase, and an upper aqueous phase. The RNA remains exclusively in the aqueous phase.

If you need to get rid of impurities to get highly purified RNA, you can transfer the aqueous phase into a clean centrifugal tube.

Add chloroform and saturated phenol (pH 4.5) in a ratio of 5:1 (v/v).

Shake for 5 sec and then spin at 13,000 rpm (12,000 x g) for 15 min at 4°C. The solution will be divided as three phases again: upper aqueous phase, middle phase and lower phase. The middle phase is not extremely visible.

3. RNA Precipitation

Transfer the aqueous phase to a fresh tube.

Add 0.7 ml of isopropyl alcohol per 1 ml of mi-Total Reagent used for homogenization.

Then shake for equal mixing and incubate samples at -20°C for 10 min and spin at 13,000 rpm (12,000 x g) for 15 min at 4°C.

The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side or bottom of the tube.

4. RNA Wash

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of mi-Total Reagent used for the initial homogenization.

Mix the sample by vortexing and spin at 13,000 rpm (12,000 x g) for 5 min at 4°C and remove the supernatant again.

5. Redissolving the RNA

At the end of the procedure, briefly dry the RNA pellet (Do not dry the RNA for too long. It may reduce the solubility). Solve the RNA in RNase-free water or TE buffer (normally in 50-100µl) and store at -70°C.

Hints and Troubleshooting

Low yield of extraction

Incomplete homogenization or lysis of samples or incomplete solution of RNA.
The RNA content varies by sample. Normally new tissue or cells contain plenty of RNA.

RNA degradation (see also notes "How to prevent RNase contamination")

Here is a list of possible causes:

1. The tissues were not immediately processed or frozen after removal from the animal.
2. Aqueous solutions or tubes were not RNase-free.
3. Cells were dispersed by trypsin digestion.
4. Samples used for isolation, or the isolated RNA preparations were not stored at the recommended -70°C .
5. Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5
6. You had forgotten to wear gloves and/or change gloves frequently during the procedure.
7. RNA contamination from spittle.

Why is there DNA contamination when extraction of total RNA?

The sample was homogenized in a too small reagent volume or the extracted solution contained solvents from the middle and lower phase.

Why is the OD260/OD280 ratio too low?

Here is a list of possible causes:

1. The RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase the absorbance at 280 nm.
2. The sample was homogenized in a too small a reagent volume.
3. Following homogenization, the samples were not stored at room temperature for 5 min to lyse completely.
4. The aqueous phase was contaminated by the phenol phase.
5. Incomplete dissolution of the final RNA pellet.