

DESCRIPTION

Total RNA Extraction Reagent is a ready-to-use kit for the isolation of total RNA from animal or plant tissue or from bacterium. The operation of this kit is very simple and easy. First, add the sample into the kit for complete lyses. Second, add chloroform into the solution and then centrifuge the sample tube. Then the homogenate will separate into form three phases: upper aqueous phase, interphase phase and organic phase. RNA remains exclusively in the aqueous phase; the RNA is recovered by precipitation with isopropyl alcohol.

APPLICATIONS

- cDNA Synthesis, cDNA library building
- Northern blot analysis, dot blot hybridization
- RNase protection assay
- RT-PCR, Quantative PCR and Real-time PCR
- Ploy(A)⁺ selection, in vitro translation

KIT CONTENTS and STORAGE

One bottle containing 100ml in a box. Store in the dark at 4 °C.

PREPARING SOLUTION BEFORE USE

- Liquid N₂ (for tissue extraction)
 - Isopropyl alcohol
 - Chloroform + 75% Ethanol (in DEPC-treated water)
 - RNase-free water 【Add diethylpyrocarbonate (DEPC) to 0.1% (v/v). Let stand overnight and autoclave.】
- Recommendation: the matched RNA kits need to be stored separately for RNA experimentation in order to avoid RNA cross-contamination.

PRECAUTION FOR PREVENTING RNase

RNases can be introduced accidentally into the RNA extraction through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent in advance. Always wear disposable gloves. Also, use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase from shared equipment.

RNA EXTRACTION PROTOCOL

Suggested sample volume of 1 micro liter (ml) of Total RNA Extraction Reagent for RNA extraction.

Sample	Maxium volume	Best volume
Tissues from animal/plant	100mg	50mg
Bacterium	1.5×10 ⁹	1×10 ⁷ -5×10 ⁸
Barn	1.5×10 ⁷	5×10 ⁶ -1×10 ⁷
Cultivated cell from animal	1.5×10 ⁷	5×10 ⁶ -1×10 ⁷
Filar epiphyte	100mg	50mg

1. HOMOGENIZATION

(1) Tissues: Grind samples into powder under the liquid N₂.

Homogenize tissue samples with this kit according to the volume recommend above.

(2) Cells Grown in Monolayer: Lyse cells directly in a culture dish by adding 1 ml of Total RNA Extraction Reagent to a 3.5 cm diameter dish, laying for 3-5 minutes, shaking 2-3 times when placing in order to lyse completely, and transfer the cell lysate into a centrifuge tube. Normally every 20-30cm² solution should add 1ml This Reagent, cell number are 5×10⁷-1×10⁷.

(3) Cells Grown in Suspension: Pellet cells by centrifugation. Lyse cells in Total RNA Extraction Reagent by repetitive pipetting. Add sample according to the volume recommended before. Washing cells before addition of Total RNA Extraction 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. After homogenization, sample can be stored at -70°C, please continue the protocol in 30 days. Incubate the homogenized samples for 15 minutes at room temp to lysis of sample completely, as normal occurrence, A little floccule won't influence the quality and yield of RNA.

Option I: Centrifuge the homogenized samples at 12,000rpm for 5 min at 4°C, Transfer the aqueous phase to a fresh tube and proceed with phase separation as described.

2. PHASE SEPARATION

Add chloroform by the ratio of 1:5 into Total RNA Extraction Reagent. Cap sample tubes securely. Shake tubes by hand and incubate them 15 minutes at ice. Centrifuge the samples at 12,000xg for 15 minutes at 4 °C. Following centrifugation, the mixture separates into an organic phase, an interphase, and an aqueous phase. RNA remains exclusively in the aqueous phase.

Option II: To get high purified RNA, you also can choose the procedure below: transfer the aqueous phase into a clean centrifugal tube, add chloroform and saturated phenol(PH4.5± 0.2) at a rate of 5:1.1(v/v/v). Shake for 15 seconds and then centrifuge at 12,000xg for 15 minutes at 4 °C. The solution will be divided as three phases again, upper aqueous phase, intergraded phase and lower phase, be ware the intergraded phase is not obvious.

3. RNA PRECIPITATION

Transfer the aqueous phase to a fresh tube. Add the same volume of isopropyl alcohol, the invert tube to mix, incubate samples at -20°C for more than 20 minutes and centrifuge at 12,000xg for 10 minutes at 4 °C. The precipitated RNA, invisible before centrifugation, forms gel-like pellet on the side or bottom of the tube.

4. RNA WASHING

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of Total RNA Extraction Reagent used for the initial homogenization. Shake the sample and try to suspend the precipitation and centrifuge at 12,000xg for 5 minutes at 4°C and remove the supernatant again.

5. DISSOLIVE RNA

Air dry the RNA pellet, do not let the RNA dry completely, it may reduce its dissolubility as this will make it difficult to resuspend. Solve RNA with RNase-free water or TE (normally 50-100ul) or Tris-HCl, stored at -70°C.

SOLUTION NOTES:

(1) When extract RNA from sample rich in protein, fat, polysaccharides etc., such as muscle, the tissue of fat and the plant stem, need to be separated for more steps. The homogenized sample needs to centrifuge at 12,000xg for 15 minutes at 4 °C. Then the fat will float on upper and need to be discarded. RNA will be left in the second phase. Transfer the clear homogenized solution into a clean tube and add chloroform, then to do the following steps.

(2) If the maximum speed of centrifugation can not meet with the requested rate, please prolong the centrifugation time.

APPENDIX

1. Yield and purity of the RNA

Absorbance analysis of yield and purity

- Prepare RNA, delute by 25mM Tris-HCl (pH7.5), RNase-free water or TE buffer in a right factor;
- Zero the spectrophotometer at 260 and 280nm with 25 mm Tris-HCl, RNase-free water or TE buffer;
- Measure the OD using 100ul the RNA diluent solution, calculate the concentration of RNA as follows: Final concentration =(Spec reading A260) × (Dilution factor) × (Conversion factor A260)

The conversion factor for RNA is 0.040ug/ul per OD260 unit

Notice: the range of Spec reading A260: 0.1<OD260<1.0

- Calculate the purity of RNA as follows:

Ratio=(Spec.readingA260)/(Spec.readingA280)

Ration of 1.8-2.0 are considered ideal purity.

(The low pH will alter the OD measurements between 260 and 280 nm, indicating a low purity)

2. Voiding DNA contamination

If genomic DNA contamination is a problem with the templates and primer of choice, one of the following strategies might help: