# ACExtract Total RNA Extraction Reagent

**Cat# CE1003 – 100 ml**

**Storage at 4℃ and keep in dark for one year**

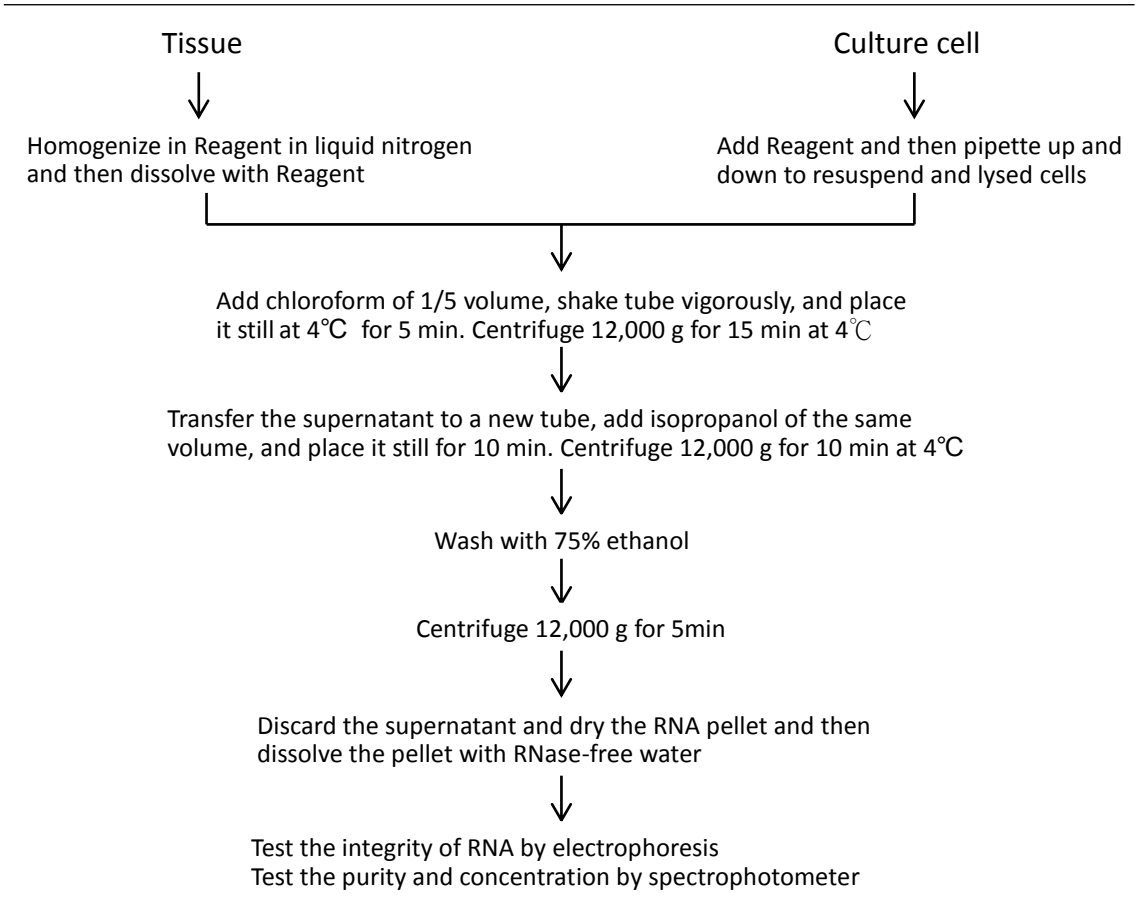
## INTRODUCTION

Based on guanidine thiocyanate and phenol, **ACExtract Total RNA Extraction Reagent** possesses extremely strong lysis capability, can lyse cell and tissue samples at short notice and timely protect the integrity of RNA. It is widely applicable to culture cells, animal tissues, microorganisms, and plant tissues with less frequent secondary metabolism, such as seedling, spire, etc. After thorough lysis of samples in RNA extraction reagent, add chloroform and centrifuge, then the liquor will form a supernatant layer, a middle layer and an organic layer (scarlet lower layer). Collect the supernatant layer which contains RNA and treat with isopropanol precipitation to get total RNA.

## CONTENTS

| No | Component | CE1003 – 100 ml |
|---|---|---|
| AA | ACExtract Total RNA Extraction Reagent | 100 ml |

## OVERVIEW



## PROTOCOL

**Note** :

1. This reagent contains phenol which is toxic and corrosive. Inhalation, contacting and swallowing may result in intoxication, burn and other bodily injuries.
2. When using this reagent, you should wear personal protective equipment, such as protective clothing, gloves, eyeshades, masks, etc.
3. In case of accidental eye contact, flush with plenty of water and go to hospital for treatment.
4. In case of skin contact, flush with plenty of polyethylene glycol 400, and go to hospital for further treatment if necessary.
5. In case of any discomfort after using this reagent, please go to hospital for treatment.

### 1. Preparation for experiment

The key to successfully extract RNA is to avoid contamination of RNases. They are ubiquitous in the environment and are extremely stable. As trace RNases can degrade RNA, protections should be taken as follows:

**a**. Wear clear disposable gloves; operate in independent and clean area; avoid talking during operation. In this case, contamination from RNA enzymes in the sweat and saliva of the experimenters can be avoided.

**b**. It is recommended to use RNase free experimental instruments, including tip and centrifuge tube. The experimental instruments should be used exclusively for RNA experiments.

**c**. Reagents used in RNA experiments should also be exclusive to avoid cross contamination caused by sharing. DEPC treated water is recommended to be stored in small packages.

### 2. Disruption and homogenization of biological samples

The maximum sample sizes which can be thoroughly lysed by 1 ml Reagent are as follows:

| | |
|---|---|
| Adherent cell [a] | 10 $cm^2$ culture area |
| Suspension cell or yeast [b] | $5\times10^6$-$10^7$ CFU |
| Bacteria | $10^7$ CFU |
| Whole blood | 50 µL |
| Animal tissue [c] | 50-100 mg |
| Plant tissue [c] | 15-30 mg |

Excessive samples may result in incomplete lysis and low purity of RNA.

**a.** Adherent cell

1) Discard the culture solution and wash with PBS for one time.
2) Add 1-2 ml Reagent in every 10 $cm^2$ culture area, and make it cover the surface of cells completely. Then pipette up and down to separate the cells down.
3) Transfer the lysis solution into a 1.5 ml centrifuge tube, and pipette up and down repeatedly till no conspicuous particulate matter exists. Place it still on ice for 5 min.

**b.** Suspension cell

1) Discard the culture solution and wash with PBS for one time.
2) Add 1 ml Reagent to every $5\times10^6$-$10^7$ cells.
3) Pipette up and down repeatedly till no conspicuous particulate matter exists. Place it still on ice for 5 min.

**c.** Animal/Plant tissue

1) Quick-freeze fresh tissues with liquid nitrogen. Quickly transfer the frozen tissues into mortars precooled by liquid nitrogen. Grind with pestle while constantly adding liquid nitrogen till the tissues are grinded into powder (Till no conspicuous particulate matter exists. Insufficient grinding will influence the yield and quality of RNA).
2) Add Reagent into the mortar thoroughly covering the powder sample. Then place it still at room temperature. After the sample melts thoroughly, continue grinding till the lysis solution is transparent.
3) Transfer the lysis solution into the centrifuge tube. Centrifuge 12,000 g at 4℃ for 5 min. Collect the supernatant.

*If liquid nitrogen is not available, try to cut up the fresh tissue and immense it into Reagent. Homogenize it at high speed with electric homogenizer and repeat the aforementioned step 3.

## 3. Extraction of total RNA

1) Add chloroform of 1/5 volume into the lysis solution. Fasten down the cover of the centrifuge tube. Shake violently with hands for 15 sec till it becomes emulsion. Place it still at 4℃ for 5 min.
2) Centrifuge 12,000 g for 5 min at 4℃.

*This step must be operated under low temperature; otherwise the products may be slightly contaminated by genome.

3) Take the centrifuge tube carefully. At this time the solution is separated into three layers: colorless upper layer, white middle layer and red lower layer. Carefully collect the upper aqueous phase and transfer it to a new centrifuge tube.

* The volume of the upper layer occupies about 60% the volume of the initial Reagent. If extracting with 1 ml Reagent, the upper aqueous phase is about 600 µl. It is recommended to recover 400-500 µl to avoid touching the middle layer which may result in genomic DNA contamination.

4) Add the isopropanol of the same volume. Mix up by shaking upside down repeatedly. Place it still at room temperature or 4℃ for 10 min.
5) Centrifuge 12,000 g at 4℃ for 10 min. Generally, white pellet can be seen.
6) Discard the supernatant carefully. Add 1 ml 75% ethanol set up with DEPC water. Place it still for 3-5 min.
7) Centrifuge 12,000 g at 4℃ for 5 min and discard the supernatant.

* To minimize the residue of impurities, it is suggested to centrifuge down briefly to collect the liquid at the bottom of the tube after discarding most of the supernatant, and then remove the liquid with pipet.

8) Dry the pellet with tube cover open at room temperature for 2-5 min. Avoid being overly dried; otherwise RNA may be hard to dissolve.

9) Add appropriate amount of DEPC water to dissolve the pellet. Pipette up and down for several times if necessary. When thoroughly dissolved, take out a small amount for testing. Store the RNA at -80℃.

## 4. RNA quality confirmation

**a.** Integrity test

1) Take 1 µl RNA, add 8 µl TE and 1 µl 10×DNA loading buffer, and mix up.

2) Carry out electrophoresis on 1% agarose gel. If three clear bands can be seen after EtBr staining, it is testified that RNA is of high integrity.

**b.** Purity and concentration test

1) Dilute RNA with TE. Detect OD value at 260 nm and 280 nm, and calculate OD260/OD280. The ratio of pure RNA is between 1.8 and 2.2. If the ratio is smaller than 1.8, it is possible that there is DNA or protein contamination; if it is smaller than 1.6, RNA may not be thoroughly dissolved.

2) RNA concentration (ng/µl)= OD 260 ×dilution fold×40

## TROUBLESHOOTING

### 1. RNA degradation.

If the user is sure that the reagents/appliances used to extract RNA are out of question, and then degradation of RNA generally occurs when the sample is homogenized. When the sample is separated from the living organism/or the original growing environment, the endogenous RNases will start to degrade RNA, the speed of which is relevant to the content of RNases and temperature. Only two methods can thoroughly restrain the activity of endogenous RNases:

1. Add the lysis solution immediately and thoroughly and rapidly homogenize it. This method is only applicable to cultures and tissues whose contents of endogenous RNases are low.

2. Tissues which are hard to be homogenized and whose content of endogenous RNases is high, such as liver, pancreas, spleen, muscles, etc., or plant tissues should be cut into small pieces and then immediately be frozen in liquid nitrogen. In the whole grinding process, make sure that the sample is not melting. Because ice crystal will be formed in the frozen sample, consequently causing damage to the internal structure of cells, this would make endogenous RNases to degrade RNA more easily. When the sample is thoroughly homogenized, Reagent can come into full contact with dissociated RNA and protect RNA from degradation.

### 2. How to store tissue sample?

If RNA cannot be immediately extracted, the tissue should be promptly put into liquid nitrogen to freeze after being separated from the body, and then be stored in liquid nitrogen or at -80℃.

Notice: fresh tissues cannot be put at -80℃ directly, or otherwise the freezing process of the specimen will be long enough for the endogenous RNase to degrade RNA.

### 3. The RNA is contaminated by DNA.

After adding chloroform, the lysis solution should be centrifuged at high speed at low temperature. RNA is drawn

into the upper aqueous phase. The middle and the lower layers are organic phases containing chloroform and DNA stays in the middle layer. Chloroform and water will dissolve with each other at room temperature at certain proportion. Therefore, Centrifuge at room temperature will result in contamination of a small amount of genomes in the upper aqueous phase. When collecting the upper liquid, be careful not to touch the middle and lower layers. It is well worth to sacrifice certain yields and leave some supernatant.

**4. No pellet can be seen after adding isopropanol and centrifuge.**

RNA concentrations may be low. After adding isopropanol, it is suggested to place the sample at 4℃ or -20℃ for 10-30 min before centrifuge. If pellet still cannot be seen, pipetting the supernatant out instead of pouring to avoid lose of pellet.

**5. How to store RNA?**

For long time storage, it is suggested to be stored at -80℃ after divided into smaller batches. It can also be stored at -20℃ for a short time but should be used as soon as possible.

6. **Failure of RT-PCR.**

RT-PCR is a multistep reaction, so the reaction system should be examined first. Under the premise that PCR and reverse transcription system is out of question, the degradation of RNA is the primary cause for the failure. Degradation of RNA generally occurs in the extraction process and the longer it is stored. A small amount of newly extracted or frozen RNA can be taken to carry out electrophoresis on 1% agarose gel to detect the integrity. Taking mammalian cell/tissue as example, intact total RNA will show three clear bands on gel, which are 28 s, 18 s, and 5 s respectively; if three bands with blurry or diffused shape can be seen, part of RNA is degraded. Then start reverse transcription immediately and increase the amount of templates properly; if only one band with very small molecular weight or no band can be seen, then RNA is totally degraded and needs to be prepared newly. In addition, the purity of RNA can also influence follow-up enzyme reactions. Reagent is based on guanidine isothiocyanate approach, whose impurities mainly come from guanidine isothiocyanate and other salts, having great impact on follow-up enzyme reactions. Therefore, it is necessary to carefully use 75% ethanol (set up with DEPC water) to wash the pellet.

## PRODUCT USE LIMITATION

These products are intended for research use only.