Total RNA Isolation Kit

Cat No. PDR03-0100(S)
Size: 100 Reactions

Description

The Total RNA Isolation Kit provides an efficient 3-step method to isolate the total RNA from the tissue, cultured animal and bacterial cells, blood, and serum. This unique reagent system ensures the total RNA with a high yield and good quality from samples of unlimited size. If a larger sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user-friendly but also highly versatile. The RNA phenol extraction is not required, and the entire procedure can be completed in 60 minutes. The total RNA is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Kit Contents

Contents	PDR03-0100	PDR03-0100S
GB Buffer 1	50 mL	4 ml
GB Buffer 2	8 mL	500 µl

Background on total RNA Isolation and Purification

The accuracy of the gene expression evaluation is influenced by the concentration and quality of the input RNA. The purity and integrity of the RNA are critical elements for the overall success of the RNA-based analyses. Starting with a low quality RNA may compromise the results of downstream applications which are often labor-intensive, time-consuming and very expensive. The integrity of the total RNA used should be examined prior to its use in the quantitative RT-PCR, microarrays and any array-based applications. RNA is often the most critical step in performing many fundamental molecular biology experiments, including the Northern analysis, nuclease protection assays, RT-PCR, RNA mapping, *in vitro* translation and cDNA library construction.

Feature

- Fast procedure delivering high-quality total RNA
- Ready-to-use RNA for high performance in any downstream application
- Consistent RNA yield from the starting material with a small amount
- Provide sufficient reagents and 3 steps to treat the samples

Application

- Molecular weight and size of DNA
- Purity of DNA required
- Downstream DNA applications
- Time flexibility
- Ease of DNA extraction technique or method
- Expense reduction

Required Sample

Cell	Required Sample
Tissue	50 mg
Cultured animal cells	5 x 10^6
Culture bacterial cells	1 x 10^9
Fresh Blood/Frozen Blood	300 µl

Required Materials

- Mortar and pestle
- RNase-free H_2O
- Isopropanol
- Chloroform
- Absolute EtOH for preparing 70% EtOH in H_2O (RNase free)
- Microcentrifuge tubes (RNase free)
- ß-mercaptoethanol
- RNase A (50 mg/ml)
- Water bath/ Dry bath

Optional requirements

For complete DNA degradation, add 2 µl of DNase I (2 KU/ml), mixed in a reaction buffer 【50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25°C】 to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

Total RNA Isolation Protocol

Sample Preparation

Tissue

1. Cut off 50 mg of the fresh tissue.
2. Grind the sample in the liquid nitrogen to a fine powder using a mortar and pestle.

Cultured Animal/Bacterial Cells

1. Transfer the cultured animal cells (up to 5 x 10^6) or bacterial culture (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube.
2. Centrifuge at 14~16,000 x g for 1 minute and pour off the majority of the supernatant (If more than 1.5 ml of bacterial culture is used, repeat this step). Use the remaining supernatant to re-suspend the pellet.

Fresh Blood/Frozen Blood

1. Collect blood in the EDTA-NA_2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 300 µl of the blood to a 1.5 ml microcentrifuge tube. If the blood sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube.

Step 1 Lysis

Tissue

1. Add 500 µl of the **GB Buffer 1** and 8 µl of the ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.

Cultured Animal and Bacterial Cells/Fresh Blood/Frozen Blood

Add 500 µl of the **GB Buffer 1** and 8 µl of the ß-mercaptoethanol to the sample and mix completely.

Serum

1. Transfer 100 µl of the serum to a 1.5 ml microcentrifuge tube.
2. Add 500 µl of the **GB Buffer 1** and 8 µl of the ß-mercaptoethanol and mix completely.

Incubate the Tissue/Cultured Animal and Bacterial Cells/Fresh Blood/Serum samples at 60ºC for 10 minutes. When using the Frozen Blood samples, incubate at 90ºC for 30 minutes. Incubate at 15~30ºC for 5 minutes.

For the Frozen Blood or Tissue (for all other samples, proceed directly to Step 2)

Centrifuge at 14~16,000 xg at 2~8ºC for 15 minutes, and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

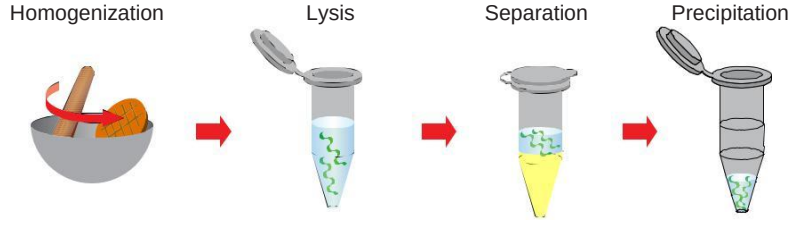
Step 2 Phase Separation

1. Add a 1/10 volume of the **GB Buffer 2** and 500 µl of the chloroform to the supernatant from the Step 1.
2. Shake vigorously and then centrifuge at 2~8ºC at 14~16,000 xg for 10 minutes. Care fully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
3. Repeat the Phase Separation Step until the interphase becomes clear, then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

NOTE: The number of repetitions is dependent on the sample type; e.g. the dense tissue samples may require a higher number of repeats.

Step 3 RNA Precipitation

1. Add 500 µl of the isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from the Step2.
2. Mix the sample by inverting gently and Incubating on the ice for 10 minutes.
3. Centrifuge at 2~8ºC at 14~16,000 xg for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% EtOH.
5. Centrifuge at 2~8ºC at 14~16,000 xg for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of the RNase-free H_2O.
   Incubate for 10 minutes at 60ºC to dissolve the pellet.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying total RNA.

Problem	Cause	Solution
Degraded RNA/ low integrity	RNases contaminant	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, EX: RNase inhibitor.
Low yields of RNA	Incomplete lysis and homogenization	Grind completely (for the tissue) Use the appropriate method for lysate preparation based on the amount of the starting materials Cut tissue samples into smaller pieces, and ensure the tissue is completely immersed in the GB Buffer 1 to achieve the optimal lysis.
	Incorrect elution conditions	Add RNase-free H_2O (50~100µL) and incubate for 10 min at 60ºC.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove EtOH in the hood briefly.

Caution

- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During the operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.