Extract Reagent (Total RNA Isolation Reagent)

Cat#: orb180503 (User Manual)

Sample: Fresh tissues (Up to 50 mg)
Cultured animal cells (Up to 5X 106)
Cultured bacterial cells (Up to 1 X 109)
Fresh blood/frozen blood (Up to 300 µl)
Serum (Up to 100 µl)
Format: Reagent form
Operation time: 15-20 minutes
Elution volume: 50-200 µl

Description

The Extract Reagent 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 proportionally, 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.

Features

- Fast procedure and 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 biology applications, including real-time RT-PCR, microarray analysis, next-generation sequencing (RNA-Seq), northern blotting, and cloning.

Kit Contents

Contents	0100	0004
ER Buffer 1	50 ml X 1 bottle	2 ml X 2 vials
ER Buffer 2	6 ml X 1 bottle	0.5 ml X 1 vial

Quality Control

The quality of the Extract Reagent is tested on a lot-to-lot basis to ensure consistent product quality

Required Materials

- Mortar and pestle
- Microcentrifuge tubes (RNase-free)
- RNase-free H2O
- ß-mercaptoethanol
- Isopropanol
- RNase A (50 mg/ml)
- Chloroform
- Water bath/ Dry bath
- Absolute ethanol for preparing 70% ethanol in H2O (RNase free)

Buffer Preparation

Optional:
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 MnCl2, 50 µg/ml BSA at 25°C to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

Extract Reagent (Total RNA Isolation Reagent) Protocol
Tissue

Sample Preparation

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

Step 1 Lysis

1. Add 500 µl of the ER 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.
3. Incubate the tissue samples at 60°C for 10 minutes.
4. Incubate at 15-30°C for 5 minutes.
5. Centrifuge at 14-16,000 x g at 2-8°C for 15 minutes, and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 2 Phase Separation

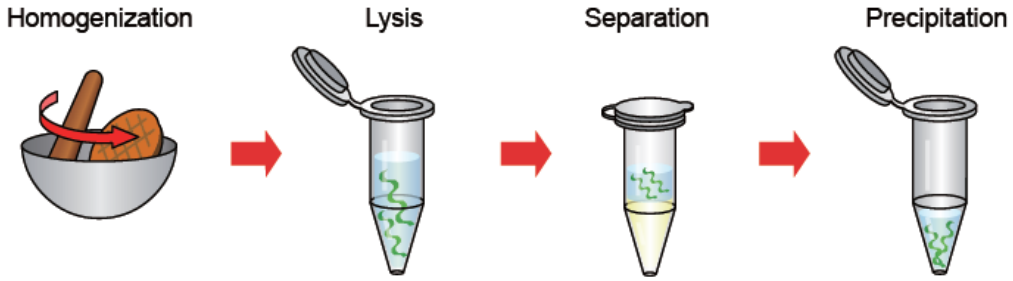
1. Add a 1/10 volume of the ER Buffer 2 and 500 µl of the chloroform to the supernatant from the Step 1.

2. Shake vigorously and then centrifuge at 2-8°C at 14-16,000 x g for 10 minutes. Carefully 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, and 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 2-8°C at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% ethanol.
5. Centrifuge at 2-8°C at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of the RNase-free H2O.
7. Incubate for 10 minutes at 60°C to dissolve the pellet.



Cultured Animal/Bacterial Cells

Sample Preparation

1. Transfer the cultured animal cells (up to 5 x 106) or bacterial culture (up to 1 x 109) 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.

Step 1 Lysis

1. Add 500 µl of the ER Buffer 1 and 8 µl of the ß-mercaptoethanol to the sample and mix completely.
2. Incubate the cultured animal and bacterial cells/fresh blood samples at 60°C for 10 minutes.
3. Incubate at 15-30°C for 5 minutes.

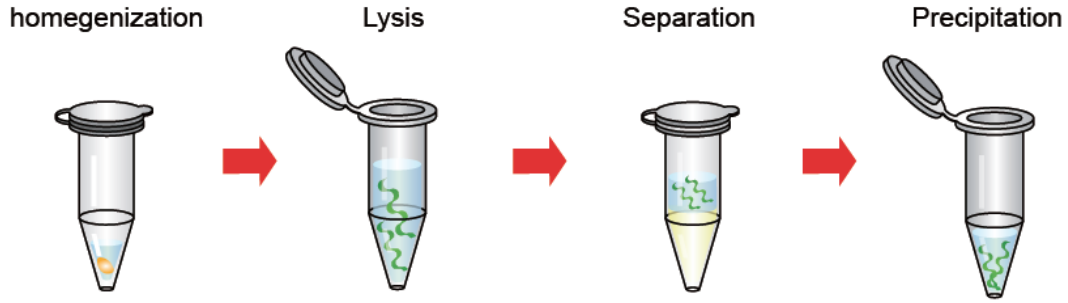
Step 2 Phase Separation

1. Add a 1/10 volume of the ER Buffer 2 and 500 µl of the chloroform to the supernatant from the Step 1.
2. Shake vigorously and then centrifuge at 2-8°C at 14-16,000 x g for 10 minutes. Carefully 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, and 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 2-8°C at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% ethanol.
5. Centrifuge at 2-8°C at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of the RNase-free H2O.
7. Incubate for 10 minutes at 60°C to dissolve the pellet.



Sample Preparation

1. Collect blood in the EDTA-NA2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer of the blood (up to 300 µl) 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

1. Add 500 µl of the ER Buffer 1 and 8 µl of the ß-mercaptoethanol to the sample and mix completely.
2. Incubate the cultured animal and bacterial cells/fresh blood samples at 60°C for 10 minutes.
3. Incubate at 15-30°C for 5 minutes.
4. For the frozen blood, Centrifuge at 14-16,000 x g at 2-8°C for 15 minutes, and transfer the supernatant to a new 1.5 ml microcentrifuge tube.

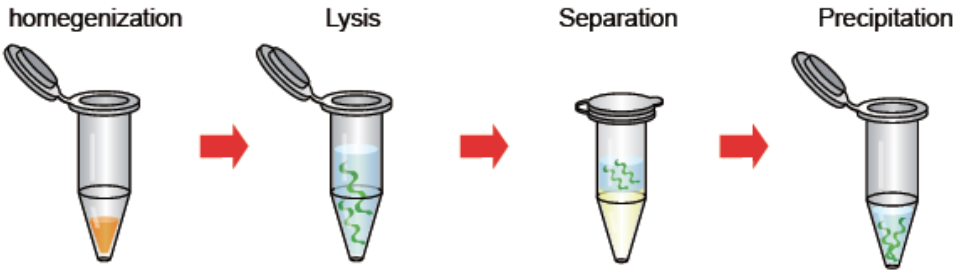
Step 2 Phase Separation

1. Add a 1/10 volume of the ER Buffer 2 and 500 µl of the chloroform to the supernatant from the Step 1.
2. Shake vigorously and then centrifuge at 2-8°C at 14-16,000 x g for 10 minutes. Carefully 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, and 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 2-8°C at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% ethanol.
5. Centrifuge at 2-8°C at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of the RNase-free H2O.
7. Incubate for 10 minutes at 60°C to dissolve the pellet.



Serum

Step 1 Lysis

1. Transfer the serum (up to 100 µl) to a 1.5 ml microcentrifuge tube.
2. Add 500 µl of the ER Buffer 1 and 8 µl of the ß-mercaptoethanol and mix completely.
3. Incubate the serum samples at60°C for 10 minutes.
4. Incubate at 15-30°C for 5 minutes

Step 2 Phase Separation

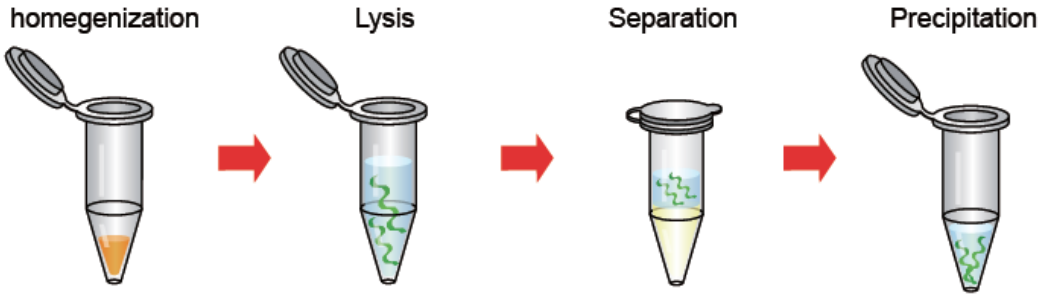
1. Add a 1/10 volume of the ER Buffer 2 and 500 µl of the chloroform to the supernatant from the Step 1.
2. Shake vigorously and then centrifuge at 2-8°C at 14-16,000 x g for 10 minutes. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.

3. Repeat the Phase Separation Step until the interphase becomes c lear, and 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 2-8°C at 14-16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% ethanol.
5. Centrifuge at 2-8°C at 14-16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of the RNase-free H2O.
7. Incubate for 10 minutes at 60°C to dissolve the pellet.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did RNA isolation with the kit.

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, e.g.: 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 ER Buffer 1 to achieve the optimal lysis
	Incorrect elution conditions	Add RNase-free H_2O (50-100 µL) and incubate for 10 min at 60°C
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purifiedRNA	Remove ethanol in the hood briefly

Related Ordering Information

Description	Size
Taq DNA polymerase	500 U
PCR SUPERMIX	100 Reactions
Hot Start SUPERMIX	100 Reactions
Ultrapure Proteinase K	100 mg
Novel Juice (Supplied in 6X Loading Buffer)	1 ml
100 bp DNA Ladder H3 RTU	500 µl
1 Kb DNA Ladder RTU	500 µl
XLarge DNA Ladder RTU	500 µl
100 mM dNTP Set	4 x 1 ml
100 mM dNTP Set	4 x 250 µl
2.5 mM dNTP Mix	1 ml

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.
- All products are for research use only.