Total RNA Isolation Kit (Blood / Cultured Cell / Fungus)

Cat No. PDC04-0100(S)
Size: 100 Reactions

Description

The **Total RNA Isolation Kit** provides a fast, simple, and cost-effective method for the isolation of total RNA from the whole blood, mammalian cells and bacterial cells. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species bases to bind to the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and the pure RNA is eluted with the REL Buffer without phenol extraction or alcohol precipitation. The RNA purified with the Total RNA Isolation Kit is suitable for a variety of routine applications, including the RT-PCR, cDNA Synthesis, Northern Blotting, Differential display, Primer Extension and mRNA selection. The entire procedure can be completed within 25-40 minutes.

Kit Contents

Contents	PDC04-0100	PDC04-0100S
Buffer RL	110 ml	4 ml
Buffer RA	45 ml	2 ml
Buffer RO	25 ml	1 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.2 ml x2)
Buffer RE	10 ml	1 ml
Column DR	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Required Sample

Cell	Required Sample
Whole blood	300 µl
mammalian cells	10^7
bacterial cells	10^9
fungus cells	10^8

Required Materials

- 1.5 ml Microcentrifuge tubes
- Absolute ethanol (96~100%)
- RNase-free pipet tips
- 14.3 M ß-mercaptoethanol
- **For the Optional Step (DNA Residue Degradation):** Add 2 µl of the DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5) and 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the final elution sample. Let it stand for 10 minutes at the room temperature.
- **For the Gram-positive bacteria sample:** lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use).
- **For the fungus sample:** lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35 mM ß-mercaptoethanol).

Total RNA Isolation Kit Protocol

Step 1 Sample Cells Harvesting

Fresh 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 sterile1.5 ml microcentrifuge tube.
3. Add 900 µl of the Buffer RL and mix by inversion.
4. Incubate the tube on ice for 10 minutes (invert twice during incubation).
5. Centrifuge for 5 minutes at 4,000 x g at 4ºC.
6. Remove the supernatant completely and resuspend the cells in 100 µl of Buffer RL by pipetting the pellet.

Cultured Mammalian Cells

1. Transfer the cultured mammalian cells (up to 10^7) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 1 minute. Remove the supernatant completely and resuspend the cells in 100 µl of the Buffer RL by pipetting the pellet.

Gram-Negative Bacterial Cells

1. Transfer the cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 200 µl of the Buffer RO by pipetting the pellet. Incubate at the room temperature for 5 minutes.

Gram-Postive Bacterial Cells

1. Transfer the cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 200 µl of the lysozyme buffer by pipetting the pellet. Incubate at the room temperature for 10 minutes.

Fungus Cells

1. Transfer the fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 5 minutes.
3. Remove the supernatant completely and resuspend the cells in 600 µl of the sorbitol Buffer by pipetting the pellet.
4. Add 200 U of the lyticase or zymolase. Incubate at 30ºC for 30 minutes.
5. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
6. Remove the supernatant completely and resuspend the cells in 200 µl of the Buffer RO by pipetting the pellet. Incubate at the room temperature for 5 minutes.

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Step 2 Lysis

Fresh Blood/Mammalian Cells

1. Add 400 µl of the Buffer RA and 4 µl of the ß-mercaptoethanol to the resuspended cells from the Step 1 and shake vigorously. Incubate at the room temperature for 5 minutes.
2. Centrifuge at 16,000 x g for 10 minutes.
3. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Bacterial Cells/ Fungus Cells

1. Add 300 µl of the Buffer RA and 3 µl of the ß-mercaptoethanol to the sample lysate from the Step 1 and mix by vortexing. Incubate at the room temperature for 5 minutes.
2. Centrifuge at 16,000 x g for 10 minutes.
3. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Binding

1. Add 500 µl of the 70% ethanol prepared with the ddH_2O (RNase-free and DNase-free) to the sample lysate from the Step 2 and shake vigorously (break up any precipitate by pipetting).
2. Place a Column DR in a Collection Tube. Apply 600µl of the mixture to the Column DR.
3. Centrifuge at 14,000 x g for 1 minute. Discard the flow-through and place the Column DR in the same Collection tube.
4. Transfer the remaining mixture to the same Column DR.
5. Centrifuge at 14,000 x g for1 minute. Discard the flow-through and place the Column DR in the same Collection tube.

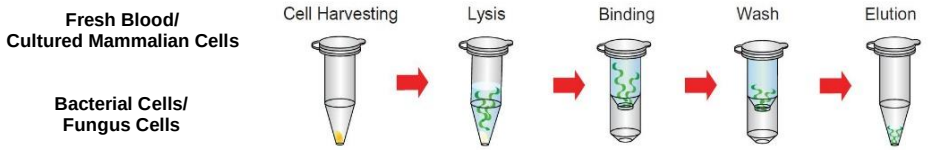
Step 4 Wash

1. Add 400 µl of the Buffer W1 into the Column DR. Centrifuge at 14,000 x g for 30 seconds.
2. Discard the flow-through and place the Column DR back into the same Collection tube.
3. Add 600 µl of the Buffer W2 (Ethanol added) into the Column DR.
4. Centrifuge at 14,000 x g for 30 seconds.
5. Discard the flow-through and place the Column DR back into the same Collection tube.
6. Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 5 Elution

1. To elute RNA, place the Column DR in a clean 1.5 ml microcentrifuge tube.
2. Add 50 ~100 µl of the Buffer RE to the center of each Column DR, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

Optional DNase treatments can be followed to remove the unwanted DNA residue



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	Use the appropriate method for the lysate preparation based on the amount of the starting materials immersed in the Buffer RA to achieve the optimal lysis.
	Incorrect elution conditions	Add 50µl of the RE Buffer to the center of each Column DR, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 14,000 x g again for 2 minutes to remove the residual W2 Buffer.

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination.
- Add 60 ml of the ethanol (96~100%) to the Buffer W2, and shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- The Buffers RA and W1 contain irritants., so please wear gloves when handling these buffers.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses