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

Cat. No.: SN017-0100 Size: 100 Reactions
Cat. No.: SN017-0004 Size: 4 Reactions
Sample: Whole blood (up to 300 µl)
Mammalian cells (up to 1 x 10^7)
Bacterial cells (up to 1 x 10^9)
Fungus Cells (up to 1 x 10^8)
Yield: Up to 30 µg
Format: Spin column
Operation time: 25-40 minutes
Elution volume: 50-200 µl

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 RE 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.

Features
- Fast procedure and delivering high-quality total RNA.
- Ready-to-use RNA for high performance in any downstream application.
- Consistent RNA yield from a small amount of starting material.

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

Kit Contents

Contents	SN017-0100	SN017-0004
Buffer RL	110 ml X 1 bottle	2 ml X 2 vials
Buffer RA	45 ml X 1 bottle	2 ml X 1 vial
Buffer RO	25 ml X 1 bottle	1 ml X 1 vial
Buffer W1	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (Add ethanol)	15 ml X 1 bottle (60 ml X 1 bottle)	0.3 ml X 2 vials (1.2 ml X 2 vials)
Buffer RE	10 ml X 1 bottle	1 ml X 1 vial
DR Column	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 2 bags	

Quality Control
The quality of the Total RNA Isolation Kit (Blood/ Cultured Cell/ Fungus) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials
- Ethanol (96-100%).
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes.
- 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 25°C} 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 zymolyase, 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 the blood (up to 300 µl) 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 4°C.
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-Positive 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.
4. 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 zymolyase.
5. Incubate at 30°C for 30 minutes.
6. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
7. 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.

Step 2 Lysis

Fresh Blood/Mammalian Cells

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

Step 3 Binding

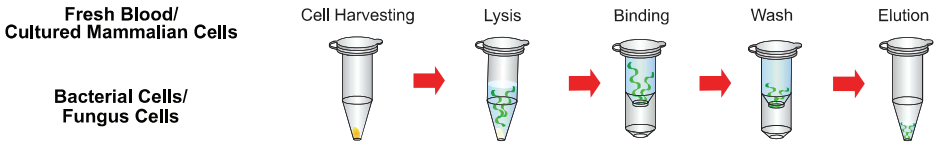
1. Add 500 µl of the 70% ethanol (prepared with the RNase-free and DNase-free ddH2O) to the sample lysate from the Step 2 and shake vigorously (break up any precipitate by pipetting).
2. Place a DR Column in a Collection Tube.
3. Apply 600 µl of the mixture to the DR Column.
4. Centrifuge at 14,000 x g for 1 minute.
5. Discard the flow-through and place the DR Column in the same Collection Tube.
6. Transfer the remaining mixture to the same DR Column.
7. Centrifuge at 14,000 x g for 1 minute.
8. Discard the flow-through and place the DR Column in the same Collection Tube.

Step 4 Wash

1. Add 400 µl of the Buffer W1 into the DR Column.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the DR Column back into the same Collection Tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the DR Column.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the DR Column back into the same Collection Tube.
7. 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 DR Column in a clean 1.5 ml microcentrifuge tube.
2. Add 50 -200 µl of the Buffer RE to the center of each DR Column, 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 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	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 50 µl of the Buffer RE to the center of each DR Column, 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.

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM200-0100	PCR SUPERMIX	100 Reactions
SM201-0100	Hot Start SUPERMIX	100 Reactions
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml
SD003-R500	100 bp DNA Ladder H3 RTU	500 µl
SD010-R500	1 Kb DNA Ladder RTU	500 µl
SD013-R500	XLarge DNA Ladder RTU	500 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml
ST046-1000	100 mM dNTP Set	4 x 250 µl
ST025-1000	2.5 mM dNTP Mix	1 ml

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- The Buffers RA and W1 contain irritants, so please wear gloves when handling these buffers.
- All products are for research use only.