User Bulletin

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Total RNA Extraction Miniprep System

Viogene Total RNA Extraction Miniprep System provides an economical method to purify total RNA from various samples such as cultured cells, tissues, and bacteria. A simple silica-membrane spin-column method can isolate total RNA without need of performing time-consuming phenol/ chloroform extraction and ethanol precipitation. Total RNA longer than 200 nucleotides are isolated, while small RNA such as 5.8S RNA, 5S RNA, and tRNA, which make up 15-20% of the total RNA, are excluded.

Downstream Application

- Northern blotting
- Ploy A^+ RNA selection
- cDNA synthesis
- RT-PCR
- NGS
- Gene editing

Product Contents

Cat. No	GR1001	GR1002
Preps	50	250
RX Buffer	36ml	200ml
WF Buffer (RNA)	30ml	150ml
WS Buffer (RNA)	15ml	45ml
RNase-free ddH_2O	1.5ml*2	15ml
RNA Mini Column	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

The kit of Total RNA Extraction Miniprep is shipped and stored at ambient temperature for up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37°C to redissolve.

Protocol:

- **Please read the following notes before starting the procedures, the procedures should be done in room temperature.**

Sample preparation:

Since the binding capacity of the Total RNA Mini Column is 50 μg of total RNA, in order to avoid exceeding the binding capacity, use the sample preparation guide listed in Table 1.

- Table 1. Sample preparation guide

Sample	Recommended amount of sample		Yield (μg)
Animal cells	NIH-3T3	1 x 10^6 cells	12
	HeLa	1 x 10^6 cells	15
	COS-7	1 x 10^6 cells	30
	LMH	1 x 10^6 cells	12

Animal tissues	Mouse/rat tissues		
	Embryo	10 mg	30
	Heart	10 mg	10
	Brain	10 mg	10
	Kidney	10 mg	35
	Liver	10 mg	45
	Spleen	10 mg	35
	Lung	10 mg	10
	Thymus	10 mg	45

Bacteria	*E. coli*	1 x 10^9 cells	45
	B. subtilis	1 x 10^9 cells	40

- **WARNING**, strong acids and oxidants (bleach, for example) should not be used together with RX buffer (because this kind of reaction would produce cyanide)!!!

Important Notes

- Add 60 (for GR1001) or 180 (for GR1002) ml of 98-100% ethanol into WS Buffer bottle when first open.
- All plasticware and containers should be treated properly to make sure RNase-free. Gloves should be worn when handling RNA.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- All centrifugation steps except cell pelleting should be done at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge.
- Some genomic DNA (and plasmid DNA, if any) will also be copurified with RNA. DNase treatment is therefore required when DNA-free RNA is desired. DNase can then be removed by phenol/chloroform extraction (refer to Protocol for "Removal of genomic DNA in eluted total RNA by DNase").
- Pipet a required volume of RX Buffer into another tube and add 10 µl β-mercaptoethanol (β-ME) per 1 ml RX Buffer before use, and store in room temperature.
- Complete disruption and homogenization of sample is essential for total RNA extraction.

Animal Tissue Protocol:

1. Add 350 µl RX Buffer (β-ME added) to 10 mg of liquid-nitrogen-frozen or fresh tissue. Disrupt and homogenize the sample by grinding and shearing using 20-G needle syringe or Viogene's Shearing Tube.

2. Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer. If using 20 mg of tissue add 700 µl of RX Buffer. If using Viogene's Shearing Tube, refer to "Application of Shearing Tube" section on the last page.

3. Centrifuge the lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.

4. Determine the final volume of the supernatant. Add an equal volume of 70% ethanol to the clear lysate and mix by vortexing.
If lysate is lost during the preparation, reduce the volume of ethanol accordingly. Do not centrifuge the ethanol added lysate.

5. Place a RNA Mini Column onto a Collection Tube. Add 700 µl of the ethanol-added sample (including any precipitate) into the column. Centrifuge for 30-60 seconds. Discard the flow-through.
Repeat this step for the rest of the sample. If some sample still retains in the column, repeat centrifugation until all samples pass the column.

6. Wash the column once with 0.5 ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

7. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
Add 60 (for GR1001) or 180 (for GR1002) ml of 98-100% ethanol into WS Buffer bottle when first open.

8. Centrifuge the column for another 3 minutes to remove ethanol residue.

9. Place the column onto a 1.5-ml RNase-free eppendorf tube (not provided). Add 30-50 µl RNase-free ddH_2O (provided) onto the center of the membrane.

For effective elution, make sure that the elution solution is dispensed onto the center of the membrane.

10. Stand the column for 1 minute, and centrifuge for 1-2 minutes to elute total RNA.

11. Store RNA at –70 °C.

Animal Cells Protocol:

1. Pellet 1 to 5 x 10^6 cells by centrifuging at 300 x g for 5 minutes. Remove all the supernatant.

2. Disrupt cells by adding 350 µl RX Buffer (β-ME added) to the cell pellet and vortex the sample. Homogenize the sample by using 20-G needle syringe or Viogene's Shearing Tube.

Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer. If using Viogene's Shearing Tube, apply the disrupted lysate to a Shearing Tube and centrifuge for 1 minute to shear genomic DNA.

3. Follow the Animal Tissue Protocol starting from Step 2.

Animal Cell Cytoplasm Protocol:

1. Prepare cytoplasm lysate.

Prepare cell lysis buffer: (provide by user) 20 mM Tris-HCl pH 8.0, 1 mM $MgCl_2$, 0.5% NP-40.Keep at 4 °C.

Only fresh cells are used for preparing cytoplasm lysate.

a. Harvest 5 x 10^6 - 1 x 10^7 cells and centrifuge at 300 x g to pellet cells.

b. Add 180 µl of cell lysis buffer to the cell pellet, resuspend and lysis cells by gentle pipetting. Incubate the lysate on ice for 5 minutes.

c. Centrifuge the lysate at 300 x g at 4°C for 3 minutes, transfer the supernatant to a new tube, and use the supernatant (lysate) in the following steps.

2. Add 600 µl of RX Buffer (β-ME added) to the lysate and mix by vortexing.

Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer.

3. Add 450 µl of 98-100% ethanol to the sample and mix by vortexing.

4. Follow the Animal Tissue Protocol starting from Step 4.

Bacteria Protocol:

1. **Pellet up to 1 x 10^9 bacterial cells by centrifuging at 5,000 x g (7,500 rpm) for 5 minutes. Remove all the supernatant.**

2. **Resuspend cells in 100 µl of TE buffer by vortexing.**

3. **Add lysozyme (provide by user) to a final concentration of 500 µg/ml for Gram-negative bacteria; 2 mg/ml for Gram-positive bacteria, and incubate at room temperature for 10 minutes.**

4. **Add 350 µl RX Buffer (β-ME added) to the sample and mix by vortexing.**

   Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of Buffer RX.

5. **Centrifuge lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.**

6. **Add 250 µl of 98-100% ethanol to the sample and mix by vortexing**.

7. **Follow the Animal Tissue Protocol starting from Step 4.**

Removal of genomic DNA in eluted total RNA by DNase

1. **Incubate total RNA with RNase-free DNase I (1 unit per µg of total RNA) in 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 50 µg/ml BSA at 37 °C for 15-30 minutes.**

2. **Remove DNase I by adding an equal volume of phenol: chloroform (1:1) and mix well. Centrifuge for 5 minutes. Transfer the upper aqueous layer to a new eppendorf tube.**

3. **Add 1/10 volume of 3 M sodium acetate (pH 5.2) and 1 volume of ice-cold isopropanol to the solution and mix well. Chill on ice for 30 minutes.**

4. **Centrifuge for 10 minutes at 4°C. Discard the supernatant. Wash the pellet twice with 1 ml of 70 % ethanol and recentrifuge.**

5. **Remove all supernatant. Air dry the RNA pellet. Redissolve RNA in RNase-free ddH_2O.**

Application of Shearing Tube

Shearing Tube is designed for simple and fast homogenization of tissue and cell lysate. The lysate is loaded into a Shearing Tube sitting in a 2-ml Collection Tube and centrifuge the tube for 1-2 minutes at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge. When collecting homogenized lysate from the Collection Tube, avoid pipetting any debris and pellet formed at the bottom of the tube.

Troubleshooting

• Little or no RNA eluted:

a. Insufficient disruption or homogenization

Reduce the amount of starting sample and perform more disruption and homogenization.

b. Clogged Total RNA column

Reduce the amount of starting sample and perform more disruption and homogenization. Centrifuge the lysate to remove insoluble materials and use the supernatant only.

c. RNA is degraded

Starting sample should be fresh or frozen in liquid nitrogen and store at –80 ℃. Improper handling of the sample or storing the sample at –20℃ will cause RNA degradation.

d. RNase contamination

Use RNase-free liquid, handling tips and tubes.

• DNA contamination:

Refer to Protocol for "Removal of genomic DNA in eluted total RNA by DNase"

• A_{260}/A_{280} ratio of eluted total RNA is low:

a. Use ddH_2O of acidic pH to dilute RNA sample for spectrophotometric analysis

Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute RNA sample.

b. DNA is copurified with RNA

Refer to Protocol for "Removal of genomic DNA in eluted total RNA by DNase”**.**