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VIOGENE

User Bulletin

Cat. #: GRD1002

Total RNA Extraction Midiprep System

Description:

Viogene Total RNA Extraction Midiprep System provides a simple and fast way to purify total RNA from various sources such as cultured cells, tissue, 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.

Kit Contents:

RX Buffer (1), WF Buffer (2), WS Buffer (2), RNase-free ddH₂O (1), Total RNA Midi Column (50) and protocol (1)

Sample preparation:

Since the binding capacity of the Total RNA Midi Column is 1mg 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

Maximum amount of sample	Recommended amount of sample		Yield (µg)
Animal cells	NIH-3T3	6 x 10 ⁷ cells	800
	HeLa	6 x 10 ⁷ cells	900
	COS-7	3 x 10 ⁷ cells	900
	LMH	7 x 10 ⁷ cells	800

Animal tissues	Mouse/rat tissues		
	Embryo	100 mg	300
	Heart	100 mg	100
	Brain	100 mg	100
	Kidney	100 mg	350
	Liver	100 mg	450
	Spleen	100 mg	350
	Lung	100 mg	100
	Thymus	100 mg	450

Bacteria	<i>E. coli</i>	1 x 10 ¹⁰ cells	650
	<i>B. subtilis</i>	1 x 10 ¹⁰ cells	400

Notes:

Please read the following notes before starting the procedures.

- All procedures should be done at room temperature (20-25 °C).
- All centrifugation is at 2,500 x g (about 3,000 rpm) in a swing-bucket centrifuge.
- All plastic ware 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.
- Add 180 ml of 98-100% ethanol to WS Buffer bottle when first open.

- Pipet a required volume of RX Buffer into another tube and add 10µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer before use.
- 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”).
- Complete disruption and homogenization of sample is essential for total RNA extraction.

Animal Tissues Protocol:

- 1. Add 3.5 ml of RX Buffer (β-ME added) to 100-200 mg of liquid-nitrogen-frozen tissue, disrupt and homogenize the sample by grinding and shearing using 20-G needle-syringe.**
Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer.
- 2. Centrifuge lysate for 3 minutes to spin down insoluble material and use only the supernatant in following steps.**
- 3. 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.
- 4. Place a Total RNA Midi Column onto a 15 ml Collection tube. Add 5 ml of the ethanol-added sample (including any precipitate) into the column. Centrifuge for 3 minutes and discard the flow-through.**
Repeat this step for the rest of the sample. If some sample still remains in the column, repeat centrifugation until all sample pass the column.

- 5. Wash the column once with 5 ml of WF Buffer by centrifuging for 3 minutes, discard the flow-through.**
- 6. Wash the column twice with 4 ml of WS Buffer by centrifuging for 3 minutes, discard the flow-through.**
Add 180 ml of 98-100% ethanol into WS Buffer bottle when first open.
- 7. Centrifuge the column for an additional 5 minutes to remove ethanol residue.**
- 8. Place the column onto a new 15 ml tube. Add 500 µl RNase-free ddH₂O (provided) onto the center of the membrane.**
For effective elution, make sure that the elution solution is dispensed onto the center of the membrane.
- 9. Stand the column for 2 minutes, and centrifuge for 5 minutes to elute total RNA.**
- 10. Store RNA at -70°C.**

Animal Cells protocol:

- 1. Harvest 3×10^7 - 7×10^7 cells and centrifuge at 300 x g to pellet cells.**
- 2. Disrupt cells by adding 3.5 ml (3×10^7 cells), 7 ml (7×10^7 cells) of RX Buffer (β-ME added), and vortexing the sample. Homogenize sample by using 20-G needle-syringe.**
Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer.
- 3. Follow the Animal Tissues 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 MgCl₂, 0.5% NP-40. Keep at 4°C.

Only fresh cells are used for preparing cytoplasm lysate.

- a. Harvest 3×10^7 - 7×10^7 cells and centrifuge at 300 x g to pellet cells.
- b. Add 1.8 ml of cell lysis buffer to cell pellet, resuspend and lysis cells by gentle pipetting, and incubate 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 following steps.

2. Add 6 ml 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 4.5 ml of 98% ethanol to the sample and mix by vortexing.

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

Bacteria protocol:

1. Pellet up to 1×10^{10} bacterial cells by centrifuging at 2,500 x g (3,000 rpm) for 5 minutes to pellet cells.

2. Resuspend cells in 1000 μl of TE buffer by vortexing.

3. Add lysozyme (provide by user) to 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 3.5 ml of RX Buffer (β-ME added) to the sample and vortex.

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

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

6. Add 2.5 ml of 98% 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 eluted total RNA with RNase-free DNase I (1 unit per μg of eluted total RNA) in 50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 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 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 ddH₂O.

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

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°C. Improper handling or the sample or storing the sample at -20 °C will cause the 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”.

A₂₆₀/A₂₈₀ ratio of eluted total RNA is low:

- a. Use ddH₂O of acidic pH to dilute RNA sample for spectrophotometric analysis**
Use 10mM Tris-HCl of pH 7.5 or TE buffer to dilute RNA sample.
- b. DNA is copurified with RNA**