Viogene-BioTek Corporation
10F, No.276, Da-Tung Rd.,Sec. 1. Shijr,
New Taipei City, Taiwan,ROC
Tel: +886-2-2647 2259 Fax: +886-2-2647 5094
www.viogene.com

VIOGENE
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User Bulletin

Total RNA Extraction Midiprep System

Viogene Total RNA Extraction Midiprep 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

Product Contents

Cat. No	GRD1001	GRD1002
Preps	10	50
RX Buffer	72ml	180ml x 2
WF Buffer (RNA)	60ml	150ml x 2
WS Buffer (RNA)	25ml	45ml x 2
RNase-free ddH_2O	7ml	30ml
Total RNA Midi Column	10	50
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Total RNA Extraction Midiprep is shipping and storage at ambient temperature up to 12 months.

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

Protocol:

❖ **Please read the following notes before starting the procedures.**

Sample preparation:

Since the binding capacity of the Total RNA Midi Column is 1 mg 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	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	*E. coli*	1 x 10^{10} cells	650
	B. subtilis	1 x 10^{10} cells	400

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

Important Notes

- Add 100 ml (for GRD1001) or 180ml (for GRD1002) 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 are done at 2,500 x g (about 3,000 rpm) in a swing-bucket centrifuge.
- 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.
- Complete disruption and homogenization of sample is essential for total RNA extraction.

Animal Tissue Protocol:

1. **Add 3.5 ml RX Buffer (β-ME added) to 100-200 mg of liquid-nitrogen-frozen or fresh 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 the lysate for 3 minutes to spin down insoluble materials and use only the supernatant in the 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. 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 sample pass the column.
5. **Wash the column once with 5 ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**
6. **Wash the column once with 7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**
   Add 100 ml (for GRD1001) or 180 ml (for GRD1002) of 98-100% ethanol into WS Buffer bottle when first open.
7. **Centrifuge the column for another 5 minutes to remove ethanol residue.**
8. **Place the column onto a new 15 ml Tube. Add 0.5 ml 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.

9. **Stand the column for 2 minute, and centrifuge for 5 minutes to elute total RNA.**

10. **Store RNA at –70 ℃.**

Animal Cells Protocol:

1. **Pellet 3 x 10^7 – 7 x 10^7 cells by centrifuging at 300 x g for 5 minutes to pellet cells. Remove all the supernatant.**

2. **Disrupt cells by adding 3.5 ml (3 x 10^7), 7 ml (7 x 10^7) of RX Buffer (β-ME added) to the cell pellet and vortex the sample. Homogenize the sample by using 20-G needle syringe.**
   Add 10 μl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer.

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 ℃.
   Only fresh cells are used for preparing cytoplasm lysate.
   a. Harvest 3 x 10^7 - 7 x 10^7 cells and centrifuge at 300 x g to pellet cells.
   b. Add 1.8 ml 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 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 98-100% 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^{10} bacterial cells by centrifuging at 2,500 x g (3,000 rpm) for 5 minutes. Remove all the supernatant.**

2. **Resuspend cells in 1 ml 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 3.5 ml 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 3 minutes to spin down insoluble materials and use only the supernatant in the following steps.**

6. **Add 2.5 ml 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 $MnCl_2$, and 50 μg/ml BSA at 37 ℃ 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℃. 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.**

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 of the sample or storing the sample at –20 °C 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”.