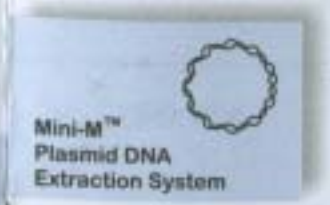
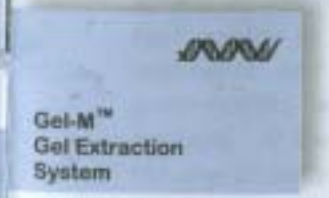
Cat. No.: GR1001 (50 preps/Kit)
GR1002 (250 preps/Kit)





Total RNA Extraction Miniprep System

User's Guide

VIOGENE

TOOLS for GENOMICS
Quality Assurance
MOLECULAR BIOLOGY CODE

For research use only

Service

Viogene regards it very important to provide satisfactory service to our every customer. In order to guarantee the best quality of our products, we value our customers' comments and suggestions on our services, or the performance, new applications, and techniques of our products. If there is any question or comment concerning the use of our products, please do not hesitate to contact our Technical Service Department by phone, e-mail, or fax, or to contact your local sales representatives. Our experienced staffs and researchers are pleased to provide you with technical help and advice. If you have problems on attaining the expected performance with our products, please contact our Technical Service Department for technical advice. If any product fails to perform properly not due to incorrect handling, please contact us or your local sales representatives for assistance.

Quality Control

We strictly require good quality control of our products by regular testing of each lot to maintain a satisfactory yield of DNA or RNA. Testing results of all lots of each product are documented. Any inquiry to access them is welcome.

Table of Contents

Description

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.

Sample Preparation Guide (Preparation Time: ~30 minutes)

Sample	Recommended amount of sample used		Maximum Yield (µg)
Animal cells	NIH-3T3	1×10^6 cells	12
	HeLa	1×10^6 cells	15
	COS-7	1×10^6 cells	30
	LMH	1×10^6 cells	12
Animal tissues (mouse/rat)	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*	1×10^9 cells	65
	B. subtilis	1×10^9 cells	40

Downstream Applications

* Northen blotting
* RT-PCR
* Poly A^+ RNA selection
* cDNA synthesis
* Primer extension
* *in vitro* translation

Product Contents

	GR1001 (50 preps) (Cat. No.)	GR1002 (250 preps) (Cat. No.)
RX Buffer	36 ml (GR1001S01)	200 ml (GR1002S01)
WF Buffer	30 ml (KR1001SWF)	150 ml (KR1002SWF)
WS Buffer	15 ml* (KR1001SWS)	45 ml** (KR1002SWS)
RNase-free ddH_2O	1.5 ml x 2	15 ml
Total RNA Mini Column (RNase-free)	50 pieces	250 pieces
Collection Tube (RNase-free)	50 pieces	250 pieces
1.5-ml Elution Tube (RNase-free)	50 pieces	250 pieces
Protocol	1	1

* For GR1001 (**50 preps**), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

** For GR1002 (**250 preps**), add 180 ml of 98-100% ethanol into WS Buffer bottle when first open.

Buffers are available for separate purchase. Please refer to the Cat. No. listed above for ordering.

Shearing Tube (RNase-free)	10 pieces/pk (GRS1001)

Shearing Tube is not provided in this system. Separate order is required.

Shipping and Storage

All components of Viogene Total RNA Extraction Miniprep System are stable at room temperature (20-25℃) for one year.

Important Notes

Please read the following notes before starting the procedures.

1. All plasticware and containers should be treated properly to make sure RNase-free. Gloves should be worn when handling RNA.

2. Buffers contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn to protect from skin contact.

3. All procedure should be done at room temperature (20-25℃).

4. 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.

5. For GR1001 (**50 preps**), add 60 ml of 98-100% ethanol into WS Buffer bottle **when first open**. For GR1002 (**250 preps**), add 180 ml of 98-100% ethanol into WS Buffer bottle **when first open**. Ethanol is provided by the user.

6. Do **not** use more than the suggested maximum amount of sample (refer to **Viogene's Hints**, No. 1, page 17).

7. Complete disruption and homogenization of sample is essential for total RNA extraction.

8. After each vortexing step, when the tube is opened, **briefly centrifuge** the tube to bring down the sample attached inside the cap to avoid generation of aerosols and contact with sample.

9. When sample or buffer is added into the column, avoid touching the rim. This is to prevent cross contamination of samples when handling the columns.

10. All centrifugation steps except cell pelleting are done at full speed (10,000 x *g* or 13,000-14,000 rpm) in a microcentrifuge.

11. 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 , page 13).

Protocol

Please refer to the Table of Contents on page 4 to choose the appropriate protocol according to the kind of sample used.

1. 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.

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

   If use 20 mg tissue, add 700 µl RX Buffer to ensure complete sample lysis.

   If Viogene's Shearing Tube is used, refer to page 13 for "**Application of Shearing Tube**".

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

   A gelatinous layer of substance may form at the bottom of the tube after centrifugation. Avoid taking it up when take out the supernatant. If this layer is not well pelleted, further centrifuge the tube for a few minutes more.

3. Determine the final volume of the supernatant. Add an equal volume of 70% ethanol to the clear lysate and mix by vortexing.

   70% ethanol should be prepared using DEPC-treated ddH_2O.

4. Place a Total 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 sample pass the column.

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

6. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

Make sure that ethanol has been added into the WS Buffer bottle when first open.

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

Residual ethanol can affect the quality of RNA and inhibit subsequent enzymatic reactions such as reverse transcriptase reaction. If necessary, centrifuge the column for a few minutes more to remove all the ethanol.

8. Place the column onto a 1.5-ml RNase-free Elution Tube. Add 30-50 µl RNase-free ddH_2O (provided) onto the membrane.

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

Eluting the column twice can result in a higher RNA recovery (refer to **Viogene's Hints**, No. 4, page 18).

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

10. Store RNA at -70℃.

II. Animal Cell Protocol

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

Any residual supernatant present will affect cell lysis by RX Buffer.

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 RX Buffer.

Add **700 µl** RX Buffer when use **1 x 10^7** cells.

If Viogene's Shearing Tube is used, refer to page 13 for "**Application of Shearing Tube**".

3. Follow the **Animal Tissue Protocol** starting from Step 2 on page 9.

III. Animal Cell Cytoplasm Protocol

1. Prepare cytoplasm lysate:

   Prepare cell lysis buffer (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.

Total RNA extracted from cytoplasm lysate are of minimum genomic DNA contamination.

   a. Pellet 5 x 10^6 to 1 x 10^7 fresh cells by centrifuging at 300 x *g* for 5 minutes. Remove all the supernatant.

   b. Add 180 µl cell lysis buffer (4℃) to the cell pellet, resuspend and lyse cells by **gentle** pipetting. Incubate the lysate on ice for 5 minutes.

   c. Centrifuge the lysate at 300 x *g* at 4℃ for 3 minutes, transfer the supernatant to a new tube and discard the pellet. Use the supernatant (lysate) in the following steps.

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

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

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

4. Follow the **Animal Tissue Protocol** starting from Step 4 on page 9.

IV. 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 TE buffer by vortexing.

3. Add lysozyme 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 5 to 10 minutes to digest the cell wall.

   Lysozyme is provided by user.

4. Add 350 µl RX Buffer to the sample and mix by vortexing.

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

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

   A gelatinous layer of substance may form at the bottom of the tube after centrifugation. Avoid taking it up when take out the supernatant. If this layer is not well pelleted, further centrifuge the tube for a few minutes more.

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

7. Follow the **Animal Tissue Protocol** starting from Step 4 on page 9.

V. Removal of genomic DNA in eluted total RNA

1. Incubate total RNA with RNase-free DNase I (1 unit DNase I per µg 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℃. Wash RNA pellet twice with 1 ml of 70% ethanol and recentrifuge.

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

VI. 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 Guide

Problem	Possible Reason	Solution
Column is clogged when passing the sample	Sample lysate contains insoluble residues or/and gelatinous substance	After sample lysis, centrifuge the sample at full speed for 5 minutes or more and only use the supernatant.
	Sample lysate is very viscous because too much sample is used	Reduce the sample amount or increase the volume of RX Buffer and ethanol proportionally.
Little or no RNA eluted	Insufficient disruption or homogenization	Reduce the amount of starting sample and perform more disruption and homogenization to the sample.
	Column is clogged	Reduce the amount of starting sample and perform more disruption and homogenization. Centrifuge the lysate to remove insoluble materials and use only the supernatant.
	RNA is not completely eluted because RNase-free ddH_2O does not penetrate into the membrane	Add ddH_2O onto the center of the membrane and stand the column for 5 minutes. If ddH_2O still retains on the membrane, pulse centrifuge the column for a few seconds to drag ddH_2O into the membrane.
	RNA is degraded	Flash freeze fresh samples in liquid nitrogen and store at -80℃ if not used immediately. Improper handling (such as thawing) of the sample or storing the sample at -20℃ will cause RNA degradation.

Problem	Possible Reason	Solution
Little or no RNA eluted	RNase contamination	Treat bench surface before use. Use RNase-free solutions, plasticware and glassware.
	No ethanol or ethanol of incorrect amount is added to the sample lysate	Determine the final volume of sample lysate obtained. Add ethanol of correct volume and concentration as indicated in the protocol.
DNA contamination	DNA is copurified with RNA	Use RNase-free DNase to treat the eluted RNA sample. DNase can then be removed by phenol/chloroform extraction (refer to **Protocol**, page 13).
		Minimize DNA copurification by extracting total RNA from cytoplasm lysate prepared from **fresh cultured cells**.
A_{260}/A_{280} ratio of eluted total RNA is low	Use ddH_2O of acidic pH to dilute RNA samples for spectrophotometric analysis	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the RNA samples (refer to **Viogene's Hints**, No. 3, page 18).
	Proteins in the sample are not completely denatured	Too much sample is used. Reduce the sample amount or increase the volume of RX Buffer and ethanol proportionally.

Problem	Possible Reason	Solution
A_{260}/A_{280} ratio of eluted total RNA is low	DNA is copurified with RNA	Refer to **Solution** section of **Problem** - "DNA contamination".
	Eluted RNA carries contaminants	Wash the column **twice** with 0.7 ml WS Buffer.
RNA appears smearing and degraded	Sample is stored and handled improperly	Flash freeze fresh samples in liquid nitrogen and store at -80℃ if not used immediately. Improper handling (such as thawing) of the sample or storing the sample at -20℃ will cause RNA degradation.
	Cell samples were harvested from an old or over-grown culture	RNA of good quality is only expected from a healthy cell or bacterial culture.
	RNase contamination	Treat bench surface before use. Use RNase-free solutions, plasticware and glassware.
	Gel electrophoresis is performed in running buffer or tank contaminated with RNase	Use fresh running buffer prepared from DEPC-treated ddH_2O and properly-cleaned tank for electrophoresis.
Poor performance in downstream applications	Eluted RNA carries ethanol residue	After wash with WS Buffer, **do** discard the flow-through, and centrifuge the column for another 3 minutes. If necessary, centrifuge the column for a few minutes more to remove all the ethanol.

Viogene's Hints

1. Low yield or purity of total RNA is usually due to incomplete lysis of the sample. Starting with a maximum amount of samples does NOT usually give the best yield of RNA. Instead, it always results in incomplete sample lysis and degradation of proteins as well as reduces RNase inactivation effectiveness of RX Buffer, thus affecting mRNA intactness and making extraction of all the total RNA from the sample unfeasible. Besides, it also requires subsequent removal of insoluble residues and yields viscous sample lysate. When the lysate is too viscous, it not only has difficulty in passing the column, but also indicates the presence of an abundant amount of contaminants such as proteins and salts. Contaminants of high amount not only affect RNA binding, but also may not be washed off completely, leading to carry over to the eluted RNA. Therefore, a good quality and yield of RNA is only expected when a sample is **completely** lysed. We advise starting with half of the maximum amount of sample suggested. When there is no problem in completely lysing the sample and passing the lysate through the column, amount of the sample to be applied can be increased gradually in the subsequent preparations.

2. Though Viogene Total RNA Extraction Miniprep System can also be used to extract total RNA from plant samples, Viogene Plant Total RNA Extraction Miniprep or Maxiprep System is recommended for plant samples for better sample lysis effect.

3. Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA and RNA samples for spectrophotometric analysis will significantly decrease A_{260}/A_{280} ratio of the sample (Wilfinger et al., 1997). 10 mM Tris-HCl of pH 7.5 or TE buffer should be used to dilute the samples.

4. When a more concentrated RNA sample is desired, elute with 30 µl RNase-free ddH_2O. Eluting the column **twice** with 30-50 µl RNase-free ddH_2O can result in a higher RNA recovery, especially when expected RNA yield is more than 30 µg. The two eluants can be collected into the same or separate tube.

Reference: Wilfinger, W. W., Mackey, K., and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**:474-481.

Viogene Products

Product	Cat. No.	Package Size	Sample	Expected Yield
Mini-M Plasmid	GF1001 GF1002	50 250	1-5 ml culture	up to 20 µg
Midi-V100 Plasmid	GDV1001 GDV1002	25 50	25-100 ml culture	up to 100 µg
Maxi-V500 Plasmid	GMV1001 GMV1002	10 25	100-250 ml culture	up to 500 µg
Blood & Tissue Genomic DNA Mini	GG1001 GG1002	50 250	200 µl whole blood 15-30 mg tissue	up to 10 µg up to 100 µg
Blood Genomic DNA Midi	GGD1001 GGD1002	20 100	1 ml whole blood	up to 50 µg
Blood Genomic DNA Maxi	GGM1001 GGM1002	10 50	5 ml whole blood	up to 300 µg
Plant Genomic DNA Mini	GPG1001 GPG1002	50 250	100 mg tissue	up to 40 µg
Plant Genomic DNA Maxi	GPGM1001	20	1 g tissue	up to 1 mg
Total RNA Mini	GR1001 GR1002	50 250	10-20 mg tissue $1x10^7$ cells	10-45 µg up to 30 µg
Total RNA Midi	GRD1001 GRD1002	10 50	0.1-0.2 g tissue $3-7x10^7$ cells	200-450 µg up to 300 µg
Total RNA Maxi	GRM1001 GRM1002	6 24	0.5-1 g tissue $2-10x10^8$ cells	1-5 mg up to 6 mg
Viral RNA Mini	GVR1001 GVR1002	50 250	150 µl body fluid	up to 90% recovery
Plant Total RNA Mini	GPR1001 GPR1002	50 250	100 mg tissue	up to 100 µg
Plant Total RNA Maxi	GPRM1001	10	1 g tissue	up to 1 mg
Gel-M Gel Extraction	EG1001 EG1002	50 250	50-200 mg agarose gel	50-80% recovery (100 bp-10 kb)
PCR-M Clean Up	PF1001 PF1002	50 250	10-100 µl DNA	up to 95% recovery (100 bp-10 kb)
VioTaq DNA Polymerase	VT1001	500 U (5 U/µl) 10X PCR Buffer containing 20 mM $MgCl_2$		
VioTwinPack Kit	VTP1001	500 U *VioTaq* DNA Polymerase (5 U/µl) 10X PCR Buffer containing 20 mM $MgCl_2$ 40 mM of dNTP mix (10 mM each)		
Clear-band Agarose	AG0050 AG0100	50 g 100 g		