



Cat. No.: GF1001 (50 preps/Kit)
GF1002 (250 preps/Kit)

Mini-M™ Plasmid DNA Extraction System

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.

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Description

Viogene Mini-M™ Plasmid DNA Extraction System provides a simple, fast, and cost-effective method to purify plasmid DNA without phenol/chloroform extraction. It is based on binding of DNA to silica-based membranes in chaotropic salts. An average yield of 1 to 20 µg of plasmid DNA can be expected from 1 to 5 ml overnight bacterial culture.

Plasmid	Culture Volume	Yield
High copy number	1.5 ml	2-8 µg
	5 ml	10-20 µg
Low copy number	1.5 ml	1-3 µg
	5 ml	5-10 µg

Preparation Time: 20-30 minutes

Downstream Applications

- * Radioactive and Fluorescent sequencing
- * Restrictive enzymatic digestion
- * Transformation
- * Ligation
- * PCR
- * Library screening or Large-scale screening

Product Contents

	GF1001 (50 preps) (Cat. No.)	GF1002 (250 preps) (Cat. No.)
MX1 Buffer*	16 ml (GF1001S01)	74 ml (GF1002S01)
MX2 Buffer	20 ml (GF1001S02)	85 ml (GF1002S02)
MX3 Buffer	21 ml (GF1001S03)	105 ml (GF1002S03)
WF Buffer	30 ml (001001SWF)	150 ml (001002SWF)
WS Buffer	15 ml** (001001SWS)	45 ml*** (001002SWS)
Elution Buffer	5 ml (001001SEB)	25 ml (001002SEB)
RNase A, 100 µg/µl	8 µl (GF1001E01)	37 µl (GF1002E01)
Mini-M™ Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces
Protocol	1	1

*Add RNase A into MX1 Buffer before use and store at 4°C (refer to **Important Notes**, No. 3, page 7)

**For GF1001 (50 preps), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

***For GF1002 (250 preps), add 180 ml of 98-100% ethanol into WS Buffer bottle when first open.

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

Shipping and Storage

All components of Viogene Mini-M™ Plasmid DNA Extraction System are stable at 20-25°C for one year. If room temperature is always above 25°C, RNase A solution is better be stored at 4°C

Important Notes

Please read the following notes before starting the procedures.

1. Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
2. All procedures should be done at room temperature (20-25°C).
3. Briefly centrifuge RNase A tube to bring down the solution. Add 1 ml of MX1 Buffer into RNase A solution and mix well. Transfer the mixture into MX1 Buffer bottle and store at 4°C.
4. If precipitation forms in MX2 Buffer, incubate at 55°C for 10 minutes to redissolve the salt precipitates. Do **not** shake MX2 Buffer, SDS present will lead to serious foaming.
5. For **GF1001 (50 preps)**, add **60 ml** of 98-100% ethanol into WS Buffer bottle **when first open**. For **GF1002 (250 preps)**, add **180 ml** of 98-100% ethanol into WS Buffer bottle **when first open**. Ethanol is provided by user.
6. All centrifugation steps are done at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge.
7. For long-term storage of the eluted plasmid, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, Elution Buffer (provided) or ddH₂O (pH 7.0-8.5) is preferred for elution of DNA immediately used for further enzymatic reactions.

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8. Centrifuge for 30-60 seconds. Discard the flow-through.
9. Wash the column once with 0.5 ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
10. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
11. Centrifuge the column at full speed for another 3 minutes to remove residual ethanol.
12. Place the column onto a new 1.5-ml centrifuge tube. Add 50 μ l of Elution Buffer (provided) onto the center of the membrane.
13. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.
14. Store plasmid DNA at 4°C or -20°C.

Ensure that ethanol has been added into WS Buffer bottle when first open.

Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions. If necessary, centrifuging the column for a few minutes more can remove all the ethanol before eluting DNA. However, do NOT remove ethanol by putting the column into an oven as high temperature may affect the intactness of the column.

ddH₂O or TE can also be used for elution (refer to **Important Notes**, No. 7, page 7).

For effective elution, make sure that the elution solution is dispensed onto the **center** of the membrane and is **completely absorbed** (refer to **Viogene's Hints**, No. 2 & 4, page 14).

If the solution still retains on the surface, **pulse-centrifuging** the tube for 1-2 seconds can drag the solution into the membrane. Do NOT over-centrifuge as the solution will get out of the membrane easily.

Protocol for Vacuum Method

1. Grow 1 to 5 ml plasmid-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.

If the bacterial cells are grown more than 16 hours, over-grown cells usually have reduced yield of plasmids (refer to **Viogene's Hints**, No. 1, page 14).

2. Pellet the cells by centrifuging for 1-2 minutes. Decant the supernatant and remove all medium residue by pipet.

Alternatively, cells can be pelleted in a culture tube using bench-top centrifuge by centrifuging at 6000 x g for 5-10 minutes.

Make sure that cells are well-pelleted in the bottom.

3. Add 250 μ l of MX1 Buffer to the pellet, and resuspend the cells **completely** by vortexing or pipetting.

Make sure that RNase A has been added into MX1 Buffer when first open.

No cell clump should be visible after resuspension of the cells. Clumped cells cannot be lysed well.

4. Add 250 μ l of MX2 Buffer and **gently** mix (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1-5 minutes.

Do NOT vortex! Vortexing shears genomic DNA and leads to chromosomal DNA contamination. If necessary, continue inverting the tube until the lysate becomes clear and viscous.

Do NOT incubate for more than **5** minutes.

5. Add 350 μ l of MX3 Buffer to neutralize the lysate, then **immediately** and **gently** mix the solution. A white precipitate should be formed.

Addition of MX3 Buffer without immediate mixing will result in uneven precipitation.

6. Centrifuge for 5-10 minutes, meanwhile insert the tip of a Mini-MTM Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*).

A compact white pellet should be formed after centrifugation.

7. Transfer the supernatant carefully into the column.

Be careful not to transfer any white pellet into the column to avoid clotting of the membrane.

8. Apply vacuum to draw all the liquid into the manifold.
9. Wash the column once with 0.5 ml WF Buffer by re-applying vacuum to draw all the liquid.
10. Wash the column once with 0.7 ml WS Buffer by re-applying vacuum to draw all the liquid.
11. Place the column onto a Collection Tube. Centrifuge the column at full speed for 3 minutes to remove residual ethanol.
12. Place the column onto a new 1.5-ml centrifuge tube. Add 50 μ l of Elution Buffer (provided) onto the center of the membrane.
13. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.
14. Store plasmid DNA at 4°C or -20°C.

Ensure that ethanol has been added into WS Buffer bottle when first open.

Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions. If necessary, centrifuging the column for a few minutes more can remove all the ethanol before eluting DNA. However, do NOT remove ethanol by putting the column into an oven as high temperature may affect the intactness of the column.

ddH₂O or TE can also be used for elution (refer to **Important Notes**, No. 7, page 7).

For effective elution, make sure that the elution solution is dispensed onto the **center** of the membrane and is **completely absorbed** (refer to **Viogene's Hints**, No. 2 & 4, page 14).

If the solution still retains on the surface, **pulse-centrifuging** the tube for 1-2 seconds can drag the solution into the membrane. Do NOT over-centrifuge as the solution will get out of the membrane easily.

* Vac-man is a trademark of Promega Corporation.

Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	Inoculate bacterial cells from a plate or a cultural stock stored for long time	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s).
	Incubation with inadequate shaking	Grow cells with vigorous shaking (e.g. 250 rpm). Adjust a suitable shaking speed according to the angular magnitude of an orbital shaker platform.
Poor cell lysis	Use too many bacterial cells harvested from a large culture or an over-grown culture	Up to 5 ml culture for high-copy plasmid Up to 10 ml culture for low-copy plasmid. When the culture is more than 5 ml, use double amount of MX1, MX2, and MX3 Buffer.
	Cell pellet is not well resuspended	Do not add MX2 Buffer until cells are completely resuspended by vortexing or pipetting.
Low yield of plasmid DNA	Not enough bacterial cells	Ensure that bacteria have grown well ($OD_{600} > 1$) after overnight incubation with vigorous shaking.
	Overgrowth of bacteria	Do not incubate for more than 16 hours.
	Plasmid does not propagate	Always inoculate bacterial cells from a freshly streaked plate and grow with required antibiotic(s).
	Inefficient or incomplete DNA elution	Efficient and complete DNA elution only takes place when elution solution is within pH 7-8.5 and is in full contact with the membrane. Make sure that no less than 30 μl of solution is dispensed onto the membrane and is completely absorbed into it before centrifugation.

Problem	Possible Reason	Solution
Low yield of plasmid DNA	Poor cell lysis	Refer to Solution section of Problem - "Poor cell lysis".
	Plasmid is larger than 10 kb	Use elution solution preheated to 70°C.
Plasmid appears smearing or degraded	Host strain is <i>endA</i> ⁺	Use <i>endA</i> ⁻ strain if possible. Or wash with WF Buffer twice . Eluting DNA with TE buffer and storing at -20°C can inhibit nuclease activity.
Genomic DNA contamination in eluate	Lysate prepared improperly	After MX2 Buffer is added, mix gently to prevent genomic DNA shearing and do not incubate for more than 5 minutes.
RNA contamination	No RNase A activity in MX1 Buffer	Ensure that RNase A is added into MX1 Buffer and stored at 4°C.
	Reduced RNase A activity in MX1 Buffer due to improper or long-time storage	Add RNase A into MX1 Buffer to a concentration of 50 µg/ml and store at 4°C.
Plasmid of poor quality	Ethanol in WS Buffer is not completely removed	After washing with WS Buffer, do discard the flow-through and centrifuge the column at full speed for 3 minutes. If necessary, centrifugation for a few minutes more can completely remove ethanol.
		1.Plasmid DNA cannot be digested well or does not deposit into the well of gel during loading
2.Plasmid is denatured and migrates faster than super-coiled form during electrophoresis	Incubate in MX2 Buffer for too long time	After MX2 Buffer is added, do not incubate for more than 5 minutes.

Viogene's Hints

1. Good yield of plasmids is always guaranteed from a healthily-grown culture. If plasmid extraction cannot be done shortly, a well-grown culture can be kept on ice for a while (<1 hour) without leading to reduction of yield. Never leave the culture on a bench for a long time as a well-grown culture starts to deteriorate due to lack of oxygen.
2. More concentrated plasmid solution can be obtained by eluting DNA in 30 μ l. However, this leads to about 40% loss of DNA due to incomplete elution. We recommend eluting in at least 50 μ l to optimize recovery. Similarly, though eluting in volume more than 50 μ l (e.g. 100 μ l) can have about 10% increase in recovery, this results in getting a more diluted plasmid solution. Each user should apply a suitable elution volume according to his/her needs.
3. Milli-Q or double-distilled H₂O stored in a laboratory for a period of time usually becomes acidic due to dissolution of CO₂ or other acidic vapor such as HCl from air. Always check the pH to make sure that it is between 7.0 to 8.5 before used. Using H₂O of pH less 7.0 for elution will lead to reduced yield of plasmid. Using H₂O of acidic pH (pH 5.0-6.0) to dilute DNA or RNA samples for spectrophotometric analysis will also significantly decrease A₂₆₀/A₂₈₀ ratio of the sample (Wilfinger *et al.*, 1997).
4. Using elution solution preheated to 70°C may increase plasmid yield by about 20%.

Reference: Wilfinger, W. W., Mackey, K., and Chomczynski, 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		Expected Yield
Mini-M Plasmid	GF1001	50	1-5 ml culture	up to 20 µg
	GF1002	250		
Midi-V100 Plasmid	GDV1001	25	25-100 ml culture	up to 100 µg
	GDV1002	50		
Maxi-V500 Plasmid	GMV1001	10	100-250 ml culture	up to 500 µg
	GMV1002	25		
Blood & Tissue Genomic DNA Mini	GG1001	50	200 µl whole blood	up to 10 µg
	GG1002	250		
Blood Genomic DNA Midi	GGD1001	20	1 ml whole blood	up to 50 µg
	GGD1002	100		
Blood Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg
	GGM1002	50		
Plant Genomic DNA Mini	GPG1001	50	100 mg tissue	up to 40 µg
	GPG1002	250		
Plant Genomic DNA Maxi	GPGM1001	20	1 g tissue	up to 1 mg
Total RNA Mini	GR1001	50	10-20 mg tissue 1x10 ⁷ cells	10-45 µg up to 30 µg
	GR1002	250		
Total RNA Midi	GRD1001	10	0.1-0.2 g tissue 3-7x10 ⁷ cells	200-450 µg up to 300 µg
	GRD1002	50		
Total RNA Maxi	GRM1001	6	0.5-1 g tissue 2-10x10 ⁸ cells	1-5 mg up to 6 mg
	GRM1002	24		
Viral RNA Mini	GVR1001	50	150 µl serum	up to 90% recovery
	GVR1002	250		
Plant Total RNA Mini	GPR1001	50	100 mg tissue	up to 100 µg
	GPR1002	250		
Plant Total RNA Maxi	GPRM1001	10	1 g tissue	up to 1 mg
Gel-M Gel Extraction	EG1001	50	50-200 mg agarose gel	50-80% recovery (100 bp-10 kb)
	EG1002	250		
PCR-M Clean Up	PF1001	50	10-100 µl DNA	up to 95% recovery (100 bp-10 kb)
	PF1002	250		
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 dNTP mix (10 mM each)		
Clear-band Agarose	AG0050	50 g		
	AG0100	100 g		