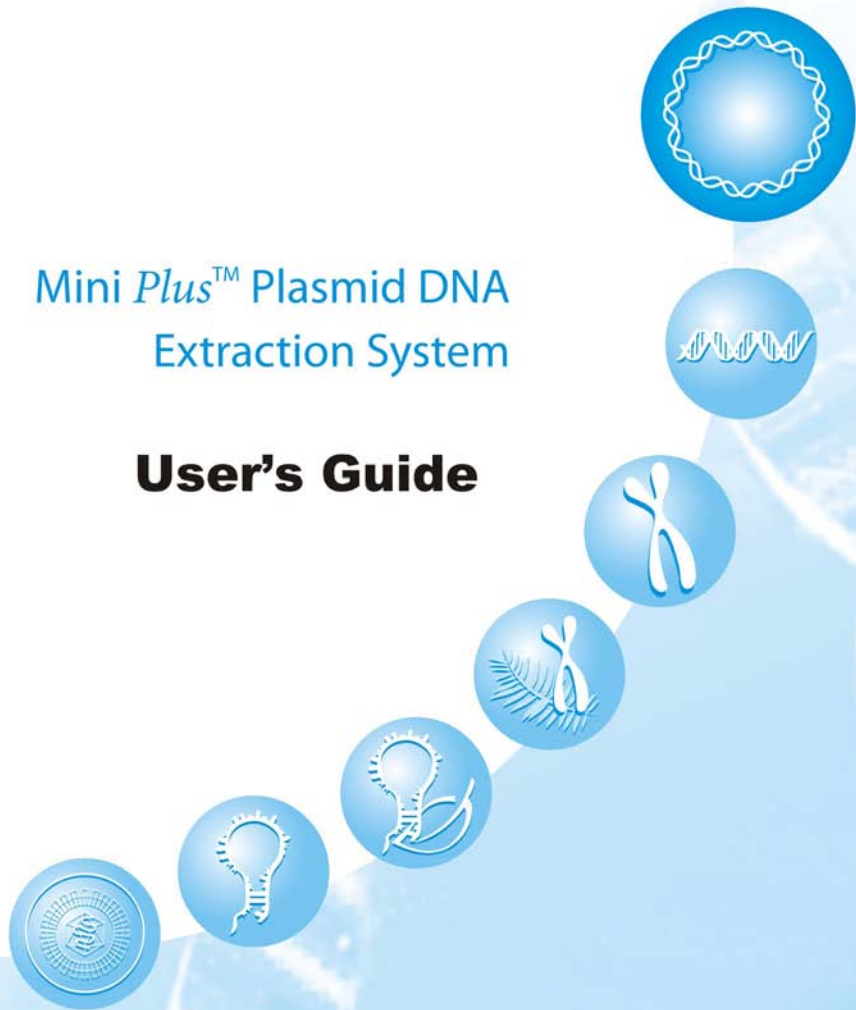




Cat No.: Mini *Plus*[™] : GF2001 (50 preps/Kit)
GF2002 (250preps/Kit)

Mini *Plus*[™] Plasmid DNA Extraction System

User's Guide



For research use only



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Service

Viogene realizes the importance in providing satisfactory service to every customer. In order to guarantee the quality of our products, we value our customers' comments and suggestions on our services, 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 staff and researchers are pleased to provide you with technical help and advice. If you have problems in attaining the expected performance with our products, please contact our Technical Service Department for technical advice. If any product fails to perform properly which is not due to incorrect handling, please contact us or your local sales representatives for assistance.

Contact Information

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Shipping and Storage

Viogene Mimi *Plus*™ Plasmid DNA Extraction System is stable at 20-25°C for one year. The kit should be stored in a dry place and kept away from direct sunlight. RNase A is preferentially stored at 4°C.

Quality Certification

Good quality control is strictly enforced 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.

Equipments and Reagents to be supplied by users

- ▶ 1.5-ml or 2-ml microcentrifuge tubes for sample collection
- ▶ Microcentrifuge with rotor for 1.5-ml and 2-ml tubes
- ▶ Ethanol (98-100%)

Must-read Notes:

Please read the following notes carefully before starting the procedures.

- ▶ Buffers provided in this system contain irritants. Appropriate safety apparel such as gloves, lab coat, or even protective goggles should be worn. People handling the kit may need suitable instruction.
- ▶ All procedures should be done at room temperature (20 ~ 25 °C) and centrifugation should be performed at 7,000 x g ~ 10,000 x g (9,000rpm ~ 13,000rpm) in a microcentrifuge, unless otherwise notified.
- ▶ 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 mix well; store at 4°C.
- ▶ If precipitation forms in MX2 Buffer, incubate at 55°C for 10 minutes to redissolve the salt precipitate. Do **not** shake MX2 Buffer, the SDS present will lead to serious foaming.
- ▶ For long-term storage of the eluted DNA, TE buffer should be used for elution. Since EDTA in TE buffer may affect downstream applications, Elution Buffer (provided) or ddH₂O (Be sure the pH of ddH₂O for elution is between 7.0 ~ 8.5!!) is preferred for elution of DNA to be immediately used for downstream enzymatic reactions.
- ▶ Please be aware that there are corresponding important notes listed below each step of the protocol. Important hints for users' references are listed beside the corresponding paragraph of the protocol. This information has been provided to help users minimize any potential problems.



Mini Plus™ Plasmid DNA Extraction System

Description

Viogene Mini Plus™ Plasmid DNA Extraction System provides a simple, fast and cost-effective method to purify plasmid DNA without phenol/chloroform extraction. This kit is optimized for increased plasmid DNA yield. It is based on binding of DNA to silica-based membranes in chaotrophic salts. An average yield of 1 to 40 µg of plasmid DNA can be expected from 1 to 5 ml overnight bacterial culture.

Parameter	Value
Average preparation time	20~30 minutes
Workable length of fragment	1.5-kbp ~ 15-kbp
Maximal recovery	95%
Minimal elution volume	30 µl
Maximal capacity	40 µg
Regular sample volume	1-5 ml

Downstream Applications

- Restrictive enzymatic digestion
- RAPD, RFLP
- PCR
- Radioactive and fluorescent sequencing
- Transformation
- Ligation
- Library screening or Large-scale screening

Product Contents:

Please check if the contents enclosed match the checklist. Record the date of first opening of each component. It is best not to pool columns or solutions from different lots. Please record the date each component is first opened on its package label.

	GF2001 (50 preps) (amount)	GF2002 (250 preps) (amount)	Check
MX1 Buffer	12 ml	60 ml	<input type="checkbox"/>
MX2 Buffer	15 ml	75 ml	<input type="checkbox"/>
MX3 Buffer	20 ml	100 ml	<input type="checkbox"/>
WN Buffer	6 ml*	30 ml**	<input type="checkbox"/>
WS Buffer	10 ml ⁺	45 ml ⁺⁺	<input type="checkbox"/>
Elution Buffer	5 ml	25 ml	<input type="checkbox"/>
Mini Plus™ Column	50 pieces	250 pieces	<input type="checkbox"/>
Collection Tube	50 pieces	250 pieces	<input type="checkbox"/>
Protocol	1	1	<input type="checkbox"/>
RNase A (20mg/ml)	0.042 ml	0.210 ml	<input type="checkbox"/>

*Add *24 ml 98-100% ethanol into WN Buffer bottle for GF2001 (50 preps) and **120 ml for GF2002 (250 preps) when first open. Ethanol is not included, and has to be provided by users. Be sure to tighten the cap following each use after the ethanol has been added.*

Add +40 ml 98-100% ethanol into WS Buffer bottle for GF2001 (50 preps) and ++180 ml for GF2002 (250 preps) when first open. Ethanol is not included, and has to be provided by users. Be sure to tighten the cap following each use after the ethanol has been added.

Buffers are available for separate purchase. Please contact us for ordering information.



Protocol for Spin 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.
 - ⚠ *Bacterial cells should not be grown more than 16 hours, and over-grown cells usually result in low plasmid and quality.*
2. Pellet the cells by centrifuging for 1-2 minutes. Decant the supernatant and remove all medium residue by pipette.
 - ⚠ Make sure that cells are well-pelleted in the bottom.
3. Add 200 μ l of MX1 Buffer to the pellet, and resuspend the cells **completely** by vortexing or pipetting.
 - ⚠ *Important!!! No cell clump should be visible after resuspension. Clumped cells lead to bad plasmid yield and quality.*
 - ⚠ Make sure that RNase A has been added into MX1 Buffer when first open.
4. Add 250 μ l of MX2 Buffer and **gently mix well** (invert the tube 8-10 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 2-5 minutes.
 - ⚠ *Important!!! The lysate should become clear and viscous. Insufficient cell-lysis leads to low plasmid yield and quality.*
 - ⚠ Do NOT vortex!!! Vortexing shears genomic DNA to contaminate plasmid DNA and leads to serious foaming.
5. Add 350 μ l of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution well. A white precipitate should be formed.
 - ⚠ Addition of MX3 Buffer without immediate mixing will result in uneven precipitation.

6. Centrifuge at 10,000 x g (13,000rpm) for 5-10 minutes, meanwhile place a Mini Plus™ Column onto a Collection Tube.
 7. Transfer the supernatant carefully into the column.
 8. Centrifuge at 7,000 x g (9,000rpm) for 30-60 seconds. Discard the flow-through.
 9. Wash the column once with 0.5 ml of WN Buffer by centrifuging at 7,000 x g (9,000rpm) for 30-60 seconds. Discard the flow-through.
 10. Wash the column once with 0.7 ml of WS Buffer by centrifuging at 7,000 x g (9,000rpm) for 30-60 seconds. Discard the flow-through.
 11. Centrifuge the column at 10,000 x g (13,000rpm) for another 3 minutes to remove residual ethanol.
 12. Place the column into a new 1.5-ml centrifuge tube. Add 50 µl of Elution Buffer (provided) onto the center of the membrane.
 13. Stand the column for 2-3 minutes, and centrifuge at 10,000 x g (13,000rpm) for 2-3 minutes to elute DNA. Store plasmid DNA at 4°C or -20°C.
- ☞ *Standing the column for a further 5 minutes may result in increased plasmid yield.*
- ☞ A compact white pellet should be formed after centrifugation
 - ☞ Be careful not to transfer any white pellet into the column to avoid clogging of the membrane.
 - ☞ Ensure that ethanol has been added into WN Buffer bottle when first open
 - ☞ Ensure that ethanol has been added into WS Buffer bottle when first open.
 - ☞ The residual ethanol in column will affect the quality of DNA and inhibit the subsequent enzymatic reactions.
 - ☞ For effective elution, make sure that the elution solution is dispensed on the center of the membrane and is completely absorbed.
 - ☞ If the solution still remains 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 run through 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.
 - ☞ *Bacterial cells should not be grown more than 16 hours, and over-grown cells usually result in low plasmid yield and quality.*
2. Pellet the cells by centrifuging for 1-2 minutes. Decant the supernatant and remove all medium residue by pipette.
 - ☞ Make sure that cells are well-pelleted in the bottom.
3. Add 200 μ l of MX1 Buffer to the pellet, and resuspend the cells **completely** by vortexing or pipetting.
 - ☞ *Important!!! No cell clump should be visible after resuspension. Clumped cells lead bad plasmid yield and quality.*
 - ☞ Make sure that RNase A has been added into MX1 Buffer when first open.
4. Add 250 μ l of MX2 Buffer and **gently mix well** (invert the tube 8-10 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 2-5 minutes.
 - ☞ *Important!!! The lysate should become clear and viscous. Insufficient cell-lysis leads to low plasmid yield and quality.*
 - ☞ Do NOT vortex!!! Vortexing shears genomic DNA to contaminate plasmid DNA and leads to serious foaming.
5. Add 350 μ l of MX3 Buffer to neutralize the lysate, then immediately and gently mix the solution well. A white precipitate should be formed.
 - ☞ Addition of MX3 Buffer without immediate mixing will result in uneven precipitation.

6. Centrifuge at 10,000 x g (13,000rpm) for 5-10 minutes, meanwhile insert the tip of a Mini Plus™ Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*).
 7. Transfer the supernatant carefully into the column. Apply Vacuum to draw all the liquid into the manifold.
 8. Wash the column once with 0.5 ml of WN Buffer by re-applying vacuum to draw all the liquid.
 9. Wash the column once with 0.7 ml of WS Buffer by re-applying vacuum to draw all the liquid.
 10. Place the column onto a Collection Tube. Centrifuge the column at 10,000 x g (13,000rpm) for another 3 minutes to remove residual ethanol.
 11. Place the column onto a new 1.5-ml centrifuge tube. Add 50 µl of Elution Buffer (provided) onto the center of the membrane.
 12. Stand the column for 2-3 minutes, and centrifuge at 10,000 x g (13,000rpm) for 2-3 minutes to elute DNA and Store at 4°C or -20°C.
- ⊕ *Standing the column for a further 5 minutes may result in increased yield of plasmid.*
- ⊘ A compact white pellet should be formed after centrifugation.
 - ⊘ Be careful not to transfer any white pellet into the column to avoid clogging of the membrane.
 - ⊘ Ensure that ethanol has been added into WN Buffer bottle when first open.
 - ⊘ Ensure that ethanol has been added into WS Buffer bottle when first open.
 - ⊘ The residual ethanol in column will affect the quality of DNA and inhibit the subsequent enzymatic reactions.
 - ⊘ For effective elution, make sure that the elution solution is dispensed on the center of the membrane and is completely absorbed.
 - ⊘ If the solution still remains 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 run through the membrane easily.



Remark

* Vac-man is a trademark of Promega Corporation.

Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	Inoculated bacterial sample from a plate or a culture stock stored over a long time period	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	Used 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, double the 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	Incubate bacterial culture with LB medium and 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).

Low yield of plasmid DNA	Inefficient or incomplete DNA elution	Make sure that elution solution is within pH 7-8.5 and fully contacts with the membrane and is absorbed. Use no less than 30µl solution to elute.
	Poor cell lysis	Refer to Solution section of problem – "Poor cell lysis".
Plasmid appears smearing or degraded	Plasmid is larger than 10-kbp	Use elution solution preheated to 70°C.
	Host strain is <i>endA</i> ⁺	Use <i>endA</i> ⁻ strain if possible. Or additionally wash with 250 µl of MX3 Buffer before WN washing.
Plasmid appears smearing or degraded	Overgrowth of bacteria	Incubate bacterial culture with LB medium and do not incubate for more than 16 hours.
	Genomic DNA contamination in elute	After MX2 Buffer added, mix gently to prevent genomic DNA shearing and do not incubate for more than 5 minutes.
RNA contamination	Lysate improperly prepared	After MX2 Buffer added, mix gently to prevent genomic DNA shearing and do not incubate for more than 5 minutes.
RNA contamination	Not enough RNase A activity in MX1 Buffer	Ensure that all RNase A is added into MX1 Buffer and stored at 4°C. After long-term storage (about 6 months), add RNase A into MX1 Buffer to the conc. 70µg/ml and store at 4°C.
Plasmid of poor quality	Ethanol in WS buffer is not completely removed	Following WS washing, discard the flow-through and centrifuge the column at full speed for 3 or more minutes.
	Use too many bacterial cells harvested from a large culture or an over-grown culture	Reduce the amount of sample used. Incubate bacterial culture with LB medium and do not incubate for more than 16 hours.



Viogene Products

Category	Product	Cat. No	Package size	Sample	Expected Yield																																																							
Mini-prep Plasmid DNA Extraction System	Mini <i>Plus</i> [™]	GF2001	50	1-5 ml culture	up to 40 µg																																																							
		GF2002	250			Ultrapure Plasmid DNA Extraction System	Midi <i>Plus</i> [™]	GDV2001	25	25-100 ml culture	more than 100 µg	GDV2002	50	Maxi <i>Plus</i> [™]	GMV2001	10	100-250 ml culture	more than 500 µg	GMV2002	25	Genomic DNA Extraction System	Blood & Tissue Genomic DNA Mini	GG1001	50	200 µl whole blood 15-30 mg tissue	up to 10 µg	GG1002	250	Blood & Tissue Genomic DNA Midi	GGD1001	20	1 ml whole blood	up to 50 µg	GGD1002	100	Blood & Tissue Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg	GGM1002	50	DNA fragment Recovery System	Gel <i>Advanced</i> [™] Gel Extraction	EG2001	50	50-200 mg agarose gel	60-90% recovery (100-bp to 10-kb), up to 20 µg	EG2002	250	PCR <i>Advanced</i> [™] PCR Clean Up	PF2001	50	10-100 µl DNA mixture	PF2002	250	Plant Genomic DNA Extraction System	Plant Genomic DNA Mini	GPG1001	50
Ultrapure Plasmid DNA Extraction System	Midi <i>Plus</i> [™]	GDV2001	25	25-100 ml culture	more than 100 µg																																																							
		GDV2002	50				Maxi <i>Plus</i> [™]	GMV2001	10	100-250 ml culture	more than 500 µg	GMV2002	25	Genomic DNA Extraction System	Blood & Tissue Genomic DNA Mini	GG1001	50	200 µl whole blood 15-30 mg tissue	up to 10 µg	GG1002		250	Blood & Tissue Genomic DNA Midi	GGD1001	20	1 ml whole blood	up to 50 µg	GGD1002	100	Blood & Tissue Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg	GGM1002	50	DNA fragment Recovery System	Gel <i>Advanced</i> [™] Gel Extraction	EG2001	50	50-200 mg agarose gel	60-90% recovery (100-bp to 10-kb), up to 20 µg		EG2002	250	PCR <i>Advanced</i> [™] PCR Clean Up	PF2001		50	10-100 µl DNA mixture	PF2002	250	Plant Genomic DNA Extraction System	Plant Genomic DNA Mini	GPG1001	50	100 mg tissue	up to 40 µg	GPG1002	250
	Maxi <i>Plus</i> [™]	GMV2001	10	100-250 ml culture	more than 500 µg																																																							
		GMV2002	25			Genomic DNA Extraction System	Blood & Tissue Genomic DNA Mini	GG1001	50	200 µl whole blood 15-30 mg tissue	up to 10 µg	GG1002	250		Blood & Tissue Genomic DNA Midi	GGD1001	20	1 ml whole blood	up to 50 µg	GGD1002		100	Blood & Tissue Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg	GGM1002	50	DNA fragment Recovery System	Gel <i>Advanced</i> [™] Gel Extraction	EG2001	50	50-200 mg agarose gel	60-90% recovery (100-bp to 10-kb), up to 20 µg	EG2002		250	PCR <i>Advanced</i> [™] PCR Clean Up	PF2001	50		10-100 µl DNA mixture	PF2002	250	Plant Genomic DNA Extraction System	Plant Genomic DNA Mini	GPG1001	50	100 mg tissue	up to 40 µg	GPG1002	250							
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		GG1002	250				Blood & Tissue Genomic DNA Midi	GGD1001	20	1 ml whole blood	up to 50 µg	GGD1002	100		Blood & Tissue Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg	GGM1002	50	DNA fragment Recovery System	Gel <i>Advanced</i> [™] Gel Extraction	EG2001	50	50-200 mg agarose gel	60-90% recovery (100-bp to 10-kb), up to 20 µg	EG2002	250		PCR <i>Advanced</i> [™] PCR Clean Up	PF2001	50	10-100 µl DNA mixture		PF2002	250	Plant Genomic DNA Extraction System	Plant Genomic DNA Mini	GPG1001	50	100 mg tissue	up to 40 µg	GPG1002	250															
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		GGD1002	100				Blood & Tissue Genomic DNA Maxi	GGM1001	10	5 ml whole blood	up to 300 µg	GGM1002	50	DNA fragment Recovery System	Gel <i>Advanced</i> [™] Gel Extraction	EG2001	50	50-200 mg agarose gel	60-90% recovery (100-bp to 10-kb), up to 20 µg	EG2002	250		PCR <i>Advanced</i> [™] PCR Clean Up	PF2001	50	10-100 µl DNA mixture		PF2002	250	Plant Genomic DNA Extraction System	Plant Genomic DNA Mini	GPG1001	50	100 mg tissue	up to 40 µg	GPG1002	250																							
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		GPG1002	250																																																									

	Plant Genomic DNA Maxi	GPGM1001	20	1 g tissue	up to 1 mg
Total RNA Extraction System	Total RNA Mini	GR1001	50	10-20 mg tissue	10-45 µg up to 30 µg
		GR1002	250	1x10 ⁷ cells	
	Total RNA Midi	GRD1001	10	0.1-0.2 g tissue	200-450 µg up to 300 µg
		GRD1002	50	3-7x10 ⁷ cells	
	Total RNA Maxi	GRM1001	6	0.5-1 g tissue	1-5 mg up to 6 mg
		GRM1002	24	2-10 x 10 ⁸ cells	
Plant Total RNA Extraction System	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
Viral RNA Extraction System	Viral RNA Mini	GVR1001	50	150 µl serum, plasma, cell culture sup.	up to 90% recovery
		GVR1002	250		
Taq DNA polymerase set	VioTaq [®] DNA polymerase	VT1001		500U (5 U / µl) 10X PCR buffer containing 20 mM MgCl ₂	
	VioTwinPack [®] Kit	VTP1001		500U (5 U / µl) 10X PCR buffer containing 20 mM MgCl ₂ 40 mM of dNTP mix (10 mM each)	
Agarose	LE Agarose	LE0005	5 g		
		LE0100	100 g		
		LE0500	500 g		

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