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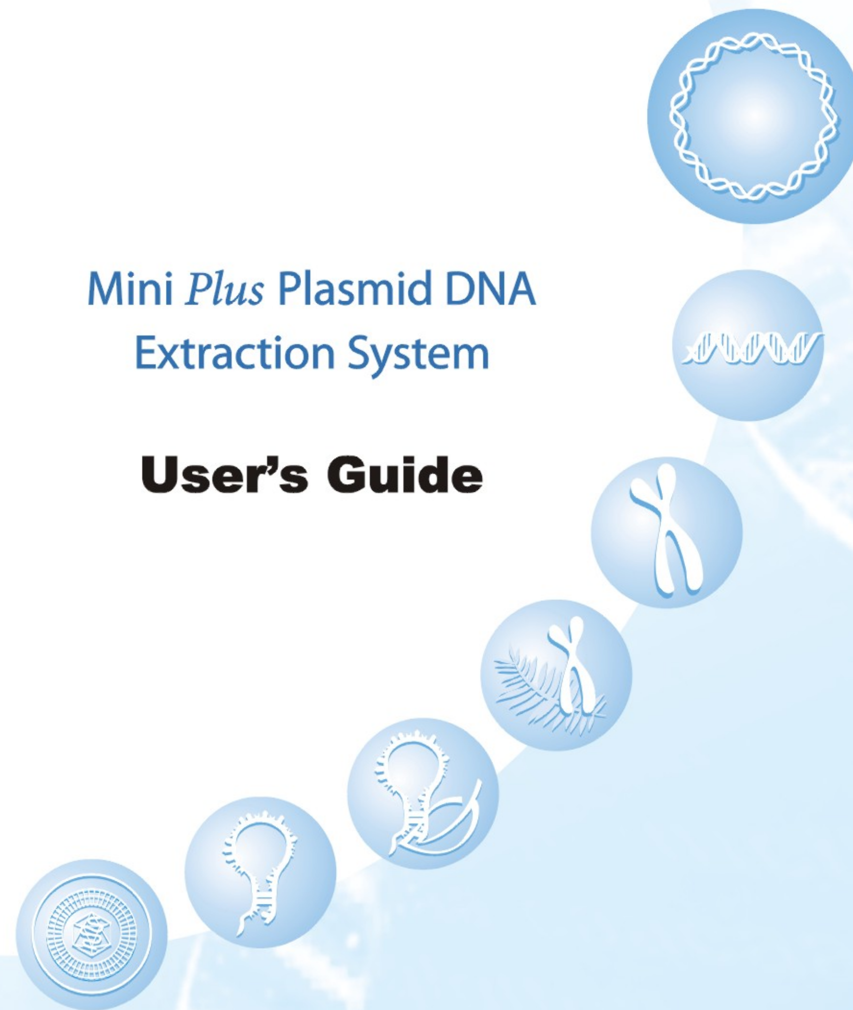
Cat No. Mini *Plus* : GF2001 (50 preps/Kit)
GF2002 (250preps/Kit)

Mini *Plus* Plasmid DNA Extraction System

User's Guide



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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

VIOGENE

http : //www.viogene.com

Tel : 886-2-2647-2259

Fax : 886-2-2647-5094

Ordering : service@viogene.com

Technical Services : service@viogene.com

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%)

Viogene's unique design:EasyLid

The EasyLid is designed to prevent contamination during the procedure.

Tip for EasyLid

Twist the arm of the cap and pull the cap to break the EasyLid.

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 is larger than 10-kbp	Use elution solution preheated to 70°C.
Plasmid appears smearing or degraded	Host strain is *endA*$^{+}$	Use *endA*$^{-}$ strain if possible. Or additionally wash with 250 µl of MX3 Buffer before WN washing.
	Overgrowth of bacteria	Incubate bacterial culture with LB medium and do not incubate for more than 16 hours.
Genomic DNA contamination in elute	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.

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).

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 ddH_2O (Be sure the pH of ddH_2O for elution is between 7.0 ~ 8.5) is preferred for elution of DNA to be immediately used for downstream enzymatic reactions.
- Please be awared 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
- NGS
- Gene editing

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 vaccum manifold (e.g. Promega's Vac-man*).
   - A compact white pellet should be formed after centrifugation.
7. Transfer the supernatant carefully into the column. Apply Vacuum to draw all the liquid into the manifold.
   - Be careful not to transfer any white pellet into the column to avoid clogging of the membrane.
8. Wash the column once with 0.5 ml of WN Buffer by re-applying vacuum to draw all the liquid.
   - Ensure that ethanol has been added into WN Buffer bottle when first open.
9. Wash the column once with 0.7 ml of WS Buffer by re-applying vacuum to draw all the liquid.
   - Ensure that ethanol has been added into WS Buffer bottle when first open.
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.
    - The residual ethanol in column will affect the quality of DNA and inhibit the subsequent enzymatic reactions.
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.
    - For effective elution, make sure that the elution solution is dispensed on the center of the membrane and is completely absorbed.
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.*
    - 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.*

   Plasmids with high quality and yield always come from good bacterial sample.

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 µl of MX1 Buffer to the pellet, and resuspend the cells completely by vortexing or pipetting.
   - *Important!!! No cell clunp 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 µl 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 µl 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.

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	☐
MX2 Buffer	15 ml	75 ml	☐
MX3 Buffer	20 ml	100 ml	☐
WN Buffer	6 ml*	30 ml**	☐
WS Buffer	10 ml+	45 ml++	☐
Elution Buffer	5 ml	25 ml	☐
Mini *Plus* Column	50 pieces	250 pieces	☐
Collection Tube	50 pieces	250 pieces	☐
Protocol	1	1	☐
RNase A (20mg/ml)	0.042 ml	0.210 ml	☐

*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.*

   Plasmids with high quality and yield always come from good bacterial sample.

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 µl of MX1 Buffer to the pellet, and resuspend the cells completely by vortexing or pipetting.
   - *Important!!! No cell clunp 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 µl 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 µl 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.

   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 clogging of the membrane.

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.

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

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.

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

11. Centrifuge the column at 10,000 x g (13,000rpm) for another 3 minutes to remove residual ethanol.

    The residual ethanol in column will affect the quality of DNA and inhibit the subsequent enzymatic reactions.

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.

    For effective elution, make sure that the elution solution is dispensed on the center of the membrane and is completely absorbed.

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.*

    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.