VIOGENE®

Cat No.: Midi *Plus*™: GDV2001 (25 preps/Kit) & GDV2002 (50preps/Kit)
Maxi *Plus*™: GMV2001 (10 preps/Kit) & GMV2002 (25preps/Kit)

Midi *Plus*™ Ultrapure Plasmid Extraction System

Maxi *Plus*™ Ultrapure Plasmid Extraction System

User's Guide

For research use only

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Service

Viogene regards it as very important to provide satisfactory service to every customer. In order to guarantee the best 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 contact your local sales representatives. Our experienced staff 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 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 Midi *Plus*™ Ultrapure Plasmid Extraction System and Maxi *Plus*™ Ultrapure Plasmid Extraction System System are stable at 20-25°C for one year. Product should be stored in a dry place and kept away from direct sunlight.

Quality Certification

We strictly enforce good quality control of our products by regular testing of each lot to maintain a satisfactory yield of DNA or RNA. Lot-to-lot testing results are documented, and any inquiry to access them is wellcome.

Equipments and Reagents to be supplied by users

- 15-ml or 50-ml centrifuge tubes for collecting the samples
- Centrifuge with rotor for 15-ml or 50-ml tubes
- 1.5-ml or 2.0-ml microcentrifuge tubes for collecting plasmid DNA
- Microcentrifuge with rotor for 1.5-ml and 2-ml tubes
- Ethanol (98-100%) and Isopropanol (99-100%)

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 done at full speed (10,000 x g or 13,000rpm), unless otherwise notified.
- Spin RNase A solution tube before use, apply all of RNase A solution into VP1 Buffer bottle and mix well to store at 4°C.
- If precipitate forms in VP2 Buffer, incubate at 55°C for 10 minutes to redissolve the salt precipitate. Do not shake VP2 Buffer, SDS presents will lead to serious foaming.
- Sit VP3 Buffer on ice before use.
- The volume of VP1-3 Buffer used in the protocol is developed for specific volume of sample culture. If starting sample culture is larger than 50/100ml (GDV/GMV), please increase the volume of VP1-3 buffer proportionally.
- 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 problem.

Midi *Plus*™ Ultrapure Plasmid Extraction System

Description

Viogene Midi *Plus*™ Ultrapure Plasmid Extraction System allows the isolation of ultrapure plasmid DNA from up to 50 ml culture. Plasmid DNA purified from Viogene's proprietary anion-exchange resin is suited for use in transfection, automated sequencing and enzymatic modification.

Parameter	Value
Average preparation time	100~130 minutes
Workable length of fragment	1.5-kbp ~ 150-kbp
Maximal recovery	99%
Minimal elution volume	5 ml
Maximal capacity	>100 µg
Regular sample volume	50 ml

Downstream Applications

- Restrictive enzymatic digestion
- Modifying enzymatic reaction
- Transfection
- Radioactive and fluorescent sequencing
- PCR
- Ligation
- Labeling Hybridization

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.

	GDV2001 (25 preps)	**GDV2002** (50 preps)	Check
VP1 Buffer	120 ml	265 ml	☐
VP2 Buffer	120 ml	265 ml	☐
VP3 Buffer	120 ml	265 ml	☐
VPN Buffer	265 ml x 2	265 ml x 4	☐
VPE Buffer	130 ml	265 ml	☐
Midi-V100™ Column	25 pieces	50 pieces	☐
Mini-M® Column	50 pieces	100 pieces	☐
Protocol	1	1	☐
RNase A (20mg/ml)	0.600 ml	1.325 ml	☐

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

Protocol for GDV2001/2002:

1. Culture plasmid-containing bacterial cells in 50 ml (high-copy-number Plasmids) or 50 ml (low-copy-number plasmids) of LB medium. Grow 12-16 hours with vigorous shaking at 37°C.

   Bacterial cells should not be grown more than 16 hours, over-grown cells usually result in low plasmid and quality.

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

   The volume of VP1-3 Buffer used in the protocol is developed for 50ml sample culture. If starting sample culture is larger than 50ml, please increase the volume of VP1-3 buffer proportionally.

2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.

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

3. Equilibrate Midi-V100™ Columns by applying 3 ml of 98-100% ethanol. Allow the column to empty by gravity flow and discard the filtrate.

   Before using column, shake the column to separate resins completely then gently knock the column to make all resins down to the bottom of the column.

4. Apply 5 ml of VPN Buffer to the Midi-V100™ Column and allow it to flow through by gravity flow and discard the filtrate.

5. Resuspend the cell pellet in 4 ml of VP1 Buffer.

   Important! No cell clump should be visible after resuspension. Clumped cells lead to low plasmid yield and quality.

   Make sure that RNase A has been added into VP1 Buffer when first open.

6. Add 4 ml of VP2 Buffer, mix gently by inverting the lysate and stand for 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 contaminating plasmids and leading to serious foaming.

7. Add 4 ml of ice-cold VP3 Buffer, mix gently by inverting.

   - Addition of VP3 Buffer without immediate mixing will result in uneven precipitation.

8. Centrifuge at 20,000 x g for 15 minutes at 4°C.

   - A compact white pellet should be formed after centrifugation.

9. Apply the supernatant with plasmid DNA to the Midi-V100™ Column and allow it to flow through by gravity flow and discard the filtrate.

10. Wash the column once with 15 ml of VPN Buffer by gravity flow and discard the filtrate.

    - If volume of bacterial sample is more than 100ml, washing the column with another 10ml of VPN will get plasmids with higher quality.

11. Apply 5 ml of VPE Buffer to elute DNA by gravity flow.

    - If plasmid is larger than 50-kbp, warm up VPE Buffer at 40°C.

12. Precipitate DNA by adding 3.5 ml (0.7 volumes) of room temperature isopropanol to the elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.

13. Wash the DNA pellet with 5 ml of room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.

14. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 100 µl or selected volume of TE or ddH_2O.

15. 〈 Optional Step〉 Some insoluble material may remain in the final product. To eliminate the insoluble material, load the dissolved DNA sample into a Mini-M® Colume (sitting in a 1.5 ml tube) and spin at full speed in a microcentrifuge for 20 seconds. Collect the eluted DNA sample in the 1.5 ml tube.

16. Store DNA at -20°C.

Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	Inoculated bacterial sample from an old plate or culture stock stored over a long time period	Always inoculate utilizing 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 batch culture or an over-grown culture	Up to 50 ml culture for high-copy plasmid. Up to 250 ml culture for low-copy plasmid. When the culture is more than 50 ml, use increased amount of VP1, VP2, and VP3 Buffer.
	Cell pellet is not well resuspended	Do **not** add VP2 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	Use **no less than 5ml** VPE Buffer to elute.
	Poor cell lysis	Refer to Solution section of problem – "Poor cell lysis".
	Plasmid is larger than 50-kbp	Use VPE Buffer preheated to 40°C.
Plasmid appears smearing or degraded	Host strain is *endA*$^+$	Use *endA*$^-$ strain.
	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 VP2 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 VP1 Buffer	Ensure that all RNase A is added into VP1 Buffer and stored at 4°C. After long-term storage (about 6 months), add RNase A into VP1 Buffer to the conc. 100µg/ml and store at 4°C.
Plasmid of poor quality	Too much salt	Wash DNA pellet after isopropanol precipitation twice with 70% ethanol at room temperature.
	Used 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.

Maxi *Plus*™ Ultrapure Plasmid Extraction System

Description

Viogene Maxi *Plus*™ Ultrapure Plasmid Extraction System allows the isolation of ultrapure plasmid DNA from up to 500 ml culture. Plasmid DNA purified from Viogene's proprietary anion-exchange resin is suited for use in transfection, automated sequencing and enzymatic modification.

Parameter	Value
Average preparation time	120~150 minutes
Workable length of fragment	1.5-kbp ~ 150-kbp
Maximal recovery	99%
Minimal elution volume	10 ml
Maximal capacity	>500 µg
Regular sample volume	100 ml

Downstream Applications

- Restrictive enzymatic digestion
- Modifying enzymatic reaction
- Transfection
- Radioactive and fluorescent sequencing
- PCR
- Ligation
- Labeling Hybridization

Product Contents:

Please check if the contents enclosed match the checklist. 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.

	GMV2001 (10 preps)	**GMV2002** (25 preps)	Check
VP1 Buffer	120 ml	265 ml	☐
VP2 Buffer	120 ml	265 ml	☐
VP3 Buffer	120 ml	265 ml	☐
VPN Buffer	225 ml x 2	265 ml x 4	☐
VPE Buffer	120 ml	265 ml	☐
Maxi-V500™ Column	10 pieces	25 pieces	☐
Mini-M® Column	20 pieces	50 pieces	☐
Protocol	1	1	☐
RNase A (20mg/ml)	0.600 ml	1.325 ml	☐

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

Protocol for GMV2001/2002:

1. Culture plasmid-containing bacterial cells in 100 ml (high-copy-number Plasmids) or 100 ml (low-copy-number plasmids) of LB medium. Grow 12-16 hours with vigorous shaking at 37°C.

   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.
   - The volume of VP1-3 Buffer used in the protocol is developed for 100ml sample culture. If starting sample culture is larger than 100ml, please increase the volume of VP1-3 buffer proportionally.

2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.

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

3. Equilibrate Maxi-V500™ Columns by applying 5 ml of 98-100% ethanol. Allow the column to empty by gravity flow and discard the filtrate.

   - Before using column, shake the column to separate resins completely then gently knock the column to make all resins down to the bottom of the column.

4. Apply 10 ml of VPN Buffer to the Maxi-V500™ Column and allow it to flow through by gravity flow and discard the filtrate.

5. Resuspend the cell pellet in 10 ml of VP1 Buffer.

   Important! No cell clump should be visible after resuspension. Clumped cells lead to low plasmid yield and poor quality DNA.

   - Make sure that RNase A has been added into VP1 Buffer when first open.

6. Add 10 ml of VP2 Buffer, mix gently by inverting the lysate and stand for 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 plasmids and leads to serious foaming.

7. Add 10 ml of ice-cold VP3 Buffer, mix gently by inverting.

   Addition of VP3 Buffer without immediate mixing will result in uneven precipitation.

8. Centrifuge at 20,000 x g for 15 minutes at 4°C.

   A compact white pellet should be formed after centrifugation.

9. Apply the supernatant to the Maxi-V500™ Column and allow it to flow through by gravity flow and discard the filtrate.

10. Wash the column once with 30 ml of VPN Buffer by gravity flow and discard the filtrate.

    If volume of bacterial sample is more than 200ml, washing the column with another 15ml of VPN will get plasmid with higher quality.

11. Apply 10 ml of VPE Buffer to elute DNA by gravity flow.

    If plasmid is larger than 50-kbp, warm up VPE Buffer at 40°C.

12. Precipitate DNA by adding 7 ml (0.7 volumes) of room temperature isopropanol to the elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.

13. Wash the DNA pellet with 10 ml of room temperature 70 % ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.

14. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 250 µl or a suitable volume of TE or ddH_2O.

15. 〈Optional Step〉 Some insoluble material may remain in the final product. To eliminate the insoluble material, load the dissolved DNA sample into a Mini-M® Colume (sitting in a 1.5 ml tube) and spin at full speed in a microcentrifuge for 20 seconds. Collect the eluted DNA sample in the 1.5 ml tube.

16. Store DNA at -20°C.

Troubleshooting Guide

Problem	Possible Reason	Solution
Poor bacterial growth	Inoculated bacterial sample from an old plate or culture stock stored over a long time period	Always inoculate utilizing 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 batch culture or an over-grown culture	Up to 100 ml culture for high-copy plasmid. Up to 500 ml culture for low-copy plasmid. When the culture is more than 100 ml, use increased amount of VP1, VP2, and VP3 Buffer.
	Cell pellet is not well resuspended	Do **not** add VP2 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	Use **no less than 10ml** VPE Buffer to elute.
	Poor cell lysis	Refer to Solution section of problem – "Poor cell lysis".
	Plasmid is larger than 50-kbp	Use VPE Buffer preheated to 40°C.
Plasmid appears smearing or degraded	Host strain is *endA*$^+$	Use *endA*$^-$ strain.
	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 VP2 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 VP1 Buffer	Ensure that all RNase A is added into VP1 Buffer and stored at 4°C. After long-term storage (about 6 months), add RNase A into VP1 Buffer to the conc. 100µg/ml and store at 4°C.
Plasmid of poor quality	Too much salt	Wash DNA pellet after isopropanol precipitation twice with 70% ethanol at room temperature.
	Used 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.

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