



Cat. No.: EG1001 (50 preps/Kit)  
EG1002 (250 preps/Kit)

## Gel-M™ Gel 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.*

## Table of Contents

<b>Contents</b>	<b>Page no.</b>
Description	5
Downstream Applications	5
Product Contents	6
Shipping and Storage	6
Important Notes	7
Protocol for Spin Method	8-9
Protocol for Vacuum Method	10-11
Troubleshooting Guide	12-13
Viogene's Hints	14
Viogene Products	15

## Description

Viogene Gel-M™ Gel Extraction System is designed to extract and purify DNA fragments from agarose gel. This system is based on binding of up to 10 µg DNA to silica-based membranes in chaotropic salts with average recoveries of 50 to 80% of 100 bp to 10 kb DNA fragments.

Preparation Time: 10-15 minutes

## Downstream Applications

- \* Ligation
- \* Labeling
- \* PCR
- \* Restrictive enzymatic digestion
- \* Modifying enzymatic reaction
- \* Radioactive and Fluorescent sequencing
- \* Hybridization

## Product Contents

	EG1001 (50 preps) (Cat. No.)	EG1002 (250 preps) (Cat. No.)
GEX Buffer	60 ml (EG1001S01)	265 ml (EG1002S01)
WF Buffer	30 ml (001001SWF)	150 ml (001002SWF)
WS Buffer	15 ml* (001001SWS)	45 ml** (001002SWS)
Elution Buffer	5 ml (001001SEB)	25 ml (001002SEB)
Gel-M™ Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces
Protocol	1	1

\*For EG1001 (50 preps), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

\*\*For EG1002 (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.*

## Shipping and Storage

Viogene Gel-M™ Gel Extraction System is stable at 20-25°C for one year.

## 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. For **EG1001 (50 preps)**, add **60 ml** of 98-100% ethanol into WS Buffer bottle **when first open**. For **EG1002 (250 preps)**, add **180 ml** of 98-100% ethanol into WS Buffer bottle **when first open**. Ethanol is provided by user.
4. All centrifugation steps are done at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge.
5. DNA can be eluted in Elution Buffer (provided), Milli-Q or double-distilled H<sub>2</sub>O, or TE buffer. Since DNA elution only takes place effectively at **pH 7.0 - 8.5**, make sure that the pH of H<sub>2</sub>O is within this range (refer to **Viogene's Hints**, No. 1, page 14). For long-term storage of the eluted DNA, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, Elution Buffer or H<sub>2</sub>O is preferred for elution of DNA immediately used for further enzymatic reactions.

## Protocol for Spin Method

1. Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.

Do NOT expose the gel to UV light for a long time as DNA will be nicked or cleaved.

**Minimize** the size of the gel slice by removing extra agarose.

2. Measure the weight of the gel slice (about **50-200 mg**) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5 ml GEX Buffer to it.

Cutting the gel slice into small pieces can facilitate dissolution.

Do NOT use more than **200 mg** gel per prep.

When agarose percentage of the gel slice is **more than 2%**, add GEX Buffer as **4 to 6 volumes** of the gel slice (100 mg=0.1 ml).

3. Incubate at 60°C for 5 to 10 minutes until the gel is completely dissolved. Invert mix the tube every 1-2 minutes during incubation. **Stop** incubation when the gel has been completely dissolved. Let the gel mixture **cool** down to room temperature.

Incubation with **mixing** can enhance gel dissolution.

If gel dissolution cannot be completed in 10 minutes, refer to **Troubleshooting Guide** on page 12.

Ensure that the gel has been completely dissolved before proceeding to step 4.

4. Place a Gel-M™ Column onto a Collection Tube. Load no more than 0.7 ml dissolved gel mixture into the column.

5. Centrifuge for 30-60 seconds. Discard the flow-through. Repeat step 4 for the rest of the mixture.

6. Wash the column once with 0.5 ml of WF Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
7. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
8. Centrifuge the column at full speed for another 3 minutes to remove residual ethanol.
9. Place the column onto a new 1.5-ml centrifuge tube. Add 30-50  $\mu$ l of Elution Buffer (provided) onto the center of the membrane.
10. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.
11. Store DNA at  $-20^{\circ}\text{C}$ .

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.

ddH<sub>2</sub>O or TE can also be used for elution (refer to **Important Notes**, No. 5, 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 & 3, 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. Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.

Do NOT expose the gel to UV light for a long time as DNA will be nicked or cleaved.

**Minimize** the size of the gel slice by removing extra agarose.

2. Measure the weight of the gel slice (about **50-200 mg**) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5 ml GEX Buffer to it.

Cutting the gel slice into small pieces can facilitate dissolution.

Do NOT use more than **200 mg** gel per prep.

When agarose percentage of the gel slice is **more than 2%**, add GEX Buffer as **4 to 6 volumes** of the gel slice (100 mg=0.1 ml).

3. Incubate at 60°C for 5 to 10 minutes until the gel is completely dissolved. Invert mix the tube every 1-2 minutes during incubation. **Stop** incubation when the gel has been completely dissolved. Let the gel mixture **cool** down to room temperature.

Incubation with **mixing** can enhance gel dissolution.

If gel dissolution cannot be completed in 10 minutes, refer to **Troubleshooting Guide** on page 12.

Ensure that the gel has been completely dissolved before proceeding to step 4.

4. Insert a Gel-M™ Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man\*). Load no more than 0.7 ml of the dissolved gel mixture into the column.
5. Apply vacuum to draw all the liquid into the manifold. Load the rest of the mixture.

6. Wash the column once with 0.5 ml of WF Buffer by re-applying vacuum to draw all the liquid.
7. Wash the column once with 0.7 ml WS Buffer by re-applying vacuum to draw all the liquid.
8. Place the column onto a Collection Tube. Centrifuge the column at full speed for 3 minutes to remove residual ethanol.
9. Place the column onto a new 1.5-ml centrifuge tube. Add 30-50  $\mu$ l of Elution Buffer (provided) onto the center of the membrane.
10. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.
11. Store DNA at  $-20^{\circ}\text{C}$ .

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.

ddH<sub>2</sub>O or TE can also be used for elution (refer to **Important Notes**, No. 5, 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 & 3, 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

<b>Problem</b>	<b>Possible Reason</b>	<b>Solution</b>
Gel slice hard to dissolve	Use high percentage agarose gel	Add GEX Buffer as 6 volumes of the gel slice > 2.5%. Incubate with mixing every 1-2 minutes until complete dissolution.
	Gel slice is too big (more than 200 mg)	Use more than one column for gel slice more than 200 mg.
Low recovery of DNA fragment	Ineffective DNA elution	DNA elution does not take place well at acidic conditions. Make sure that ddH <sub>2</sub> O used is of pH between 7.0 and 8.5.
	Incomplete DNA elution	Complete DNA elution only takes place when elution solution is in full contact with the membrane. Make sure that <b>no less than 30 µl</b> of solution is dispensed onto the membrane and is completely absorbed into it before centrifugation.
	TAE or TBE buffer is repeatedly used for many times or of incorrect pH	pH of repeatedly used TAE or TBE buffer usually gets increased. Use fresh TAE or TBE buffer each time.
	Overload the column with too much agarose	<b>Higher</b> recovery is attained when <b>lower</b> amount of agarose gel is present. Minimize the size of the gel slice by removing extra gel. When gel slice is more than 200 mg, use more than one column.

<b>Problem</b>	<b>Possible Reason</b>	<b>Solution</b>
Low recovery of DNA fragment	Size of DNA fragment is more than 5 kb	Use elution solution preheated to 60°C.
Poor performance in downstream applications	Eluted DNA carries ethanol residue	Wash the column twice with 0.7 ml WS Buffer.  After wash with WS Buffer, <b>do</b> discard the flow-through, and centrifuge the column for another 3 minutes. If necessary, centrifugation for a few minutes more can completely remove ethanol. However, do <b>not</b> remove ethanol by putting the column into an oven as high temperature may affect the intactness of the column.
Non-specific DNA fragment appears in eluted DNA	DNA fragment is denatured and becomes single-stranded.  Scalpel or razor blade used to excise the gel is contaminated with other DNA fragments	To re-anneal the single-stranded DNA, incubate the tube at 95°C for 2 minutes and let it cool slowly to room temperature. Re-annealed DNA fragments are applicable for all downstream applications.  Use a new or clean scalpel or razor blade to excise the gel.

## Viogene's Hints

1. Milli-Q or double-distilled H<sub>2</sub>O stored in a laboratory for a period of time usually becomes acidic due to dissolution of CO<sub>2</sub> 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. Use H<sub>2</sub>O of pH less 7.0 for elution will lead to reduced yield of DNA. Use H<sub>2</sub>O of acidic pH (pH 5.0-6.0) to dilute DNA or RNA samples for spectrophotometric analysis will also significantly decrease A<sub>260</sub>/A<sub>280</sub> ratio of the sample (Wilfinger et al., 1997).
2. Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice, e.g., with 30 µl H<sub>2</sub>O or buffer, yields more DNA in total than eluting once with 60 µl H<sub>2</sub>O or buffer.
3. Use of elution solution preheated to 60°C can increase recovery of DNA fragment larger than 5 kb.

**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	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 <sup>7</sup> cells	10-45 µg up to 30 µg
Total RNA Midi	GRD1001 GRD1002	10 50	0.1-0.2 g tissue 3-7x10 <sup>7</sup> cells	200-450 µg up to 300 µg
Total RNA Maxi	GRM1001 GRM1002	6 24	0.5-1 g tissue 2-10x10 <sup>8</sup> cells	1-5 mg up to 6 mg
Viral RNA Mini	GVR1001 GVR1002	50 250	150 µl serum	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)
<i>VioTaq</i> DNA Polymerase	VT1001	500 U (5 U/µl) 10X PCR Buffer containing 20 mM MgCl <sub>2</sub>		
<i>VioTwinPack</i> Kit	VTP1001	500 U <i>VioTaq</i> DNA Polymerase (5 U/µl) 10X PCR Buffer containing 20 mM MgCl <sub>2</sub> 40 mM of dNTP mix (10 mM each)		
Clear-band Agarose	AG0050 AG0100	50 g 100 g		