VIOGENE

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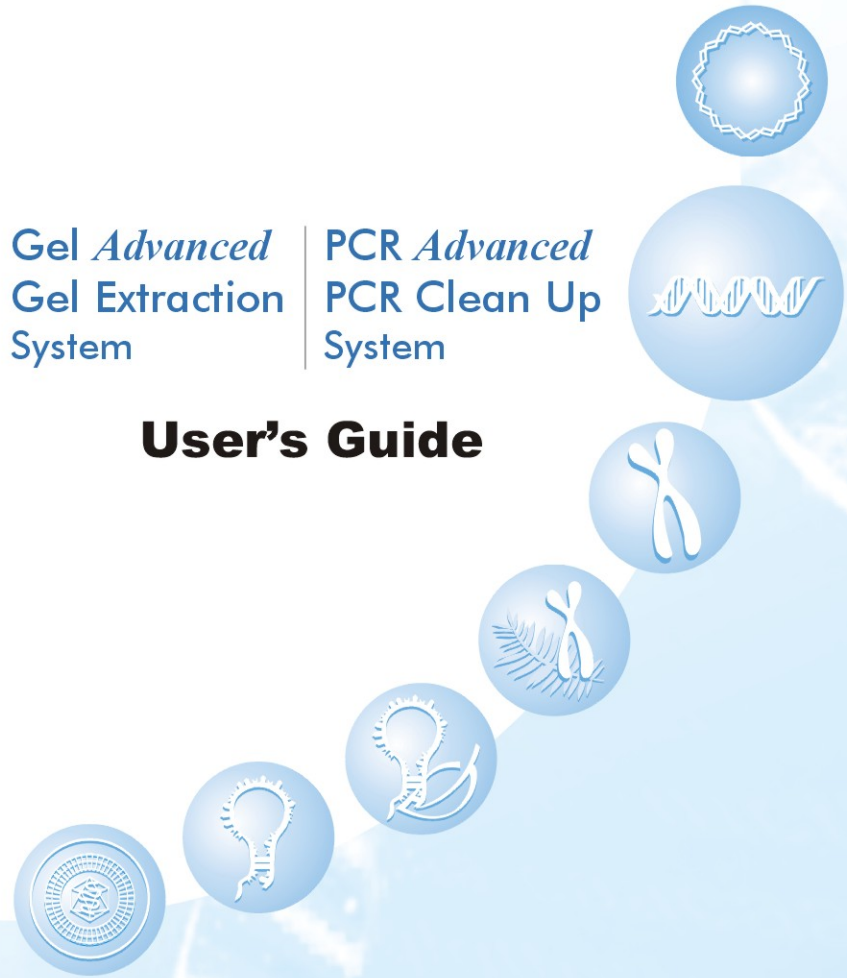
VIOGENE®

Cat No.: Gel *Advanced* : EG2001 (50 preps/Kit) & EG2002 (250preps/Kit)
PCR *Advanced* : PF2001 (50 preps/Kit) & PF2002 (250preps/Kit)

Gel *Advanced*
Gel Extraction
System

PCR *Advanced*
PCR Clean Up
System

User's Guide



For research use only

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Service

Viogene regards that it is 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, product performance, new applications, and techniques of our products. If there are any questions or comments 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 also contact our Technical Service Department for technical advice.

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 **Gel** *Advanced* Gel Extraction System and **PCR** *Advanced* PCR Clean Up System are stable at 20-25°C for one year. Product should be stored in 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. Testing results of all lots of each product are documented.

Equipments and Reagents to be supplied by users

- 1.5-ml or 2-ml microcentrifuge tubes for collecting the samples
- Clean razor blade to cut the gel (for **Gel** *Advanced*)
- UV light box for observing and managing the gel with DNA
- 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 recovery of DNA fragment	Incomplete DNA elution	Complete DNA elution only takes place when elution solution is in full contact with membrane. Make sure that at least 15 µl of solution is dispensed onto the center of the membrane and is completely absorbed before elution.
Poor performance in downstream applications	Eluted DNA carries ethanol residue	After washing with WS buffer, discard and centrifuge the column for another 3 minutes. If necessary, stand the column at room temperature for a few minutes before eluting DNA. However, do not remove ethanol by baking the column in an oven as the high temperature may affect the intactness of the column.
Poor OD_{260}/ OD_{280} ratio	Use of H_2O of acidic pH to dilute the eluted DNA	Make sure the H_2O has the pH value between 7.0~8.5

- If there are problems unable to be solved according to the roubleshooting guide, please contact your local distributor for technical support or mailing to us directly at service @viogene.com will also ensure quick response from the dedicated Technical Services Team.

Troubleshooting Guide

Problem	Possible Reason	Solution
Low recovery of DNA fragment	DNA solution used is more than 100 µl	Aliquot the sample into two or more columns. If DNA to be cleaned up is relatively diluted, more than 100 µl solution can be used per column. Add 5 times PX Buffer for each 1 µl extra DNA solution (e.g. add 600 µl PX Buffer to 120 µl DNA solution).
	Ineffective DNA elution	DNA elution does not take place well at acidic conditions. Make sure that water or buffer is of pH between 7.0 and 8.5
	Size of DNA product is more than 5-kb	Use elution solution preheated to 60°C.
	Eluted DNA carries salt residue	Wash the column twice with 0.5ml WS buffer.

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 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) in a microcentrifuge, unless otherwise notified.
- 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 the elution of DNA for immediately used of 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 problem.

Gel *Advanced* Gel Extraction System

Description

Viogene **Gel** ***Advanced*** Gel Extraction System is designed to extract and purify DNA fragments from agarose gel. This system is based on binding of up to 20μg DNA to silica-based membranes in chaotropic salts with high recoveries from 60% to 90% of 100-bp to 10-kb DNA fragments.

Parameter	Value
Average preparation time	10~15 minutes
Workable length of fragment	100-bp ~ 10-kb
Maximal recovery	60~90%
Minimal elution volume	15 μl
Maximal capacity	Up to 20 μg
Regular sample volume	50~200 mg

Downstream Applications

- Restriction enzyme digestion
- Modifying enzymatic reaction
- Radioactive and fluorescent labeling
- PCR & qPCR
- Ligation
- Labeling & hybridization
- NGS
- Gene editing

6. Place the column onto a Collection Tube. Centrifuge the column at full speed for 3 minutes to remove residual ethanol.
   - *DO NOT remove ethanol by baking the column in an oven, as high temperatures may affect the intactness of the column.*

   Residual ethanol may affect the quality of DNA and may interfere subsequent enzymatic reactions or sequencing. If necessary, stand the column at room temperature for a few minutes before eluting DNA.

7. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 μl of Elution Buffer (provided)onto the center of the membrane.
   - *Use of elution solution preheated to 60°C can increase the recovery of DNA fragments larger than 5-kb.*

   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.

8. Stand the column for 3 minutes and then centrifuge for 1-2 minutes to elute DNA. Store DNA at -20°C.

   Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30 μl H_2O or buffer, yields more DNA in total than eluting once with 60 μl H_2O or buffer.

Protocol for Vacuum Method:

1. Pipet 10-100 ul PCR product or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.

   Make sure that NO MINERAL OIL is taken. Any mineral oil will adversely interfere with the following protocol.

2. Insert a GP column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*). Add all the mixture from step 1 into the column.

   If the volume of mixture is more than 0.7 ml, load and filter 0.7 ml at each time until all the mixture has been filtrated.

3. Apply the vacuum to draw all the liquid into the manifold.

4. Wash the column once with 0.5ml of WN Buffer by re-applying the vacuum to draw all the liquid.

   Ensure that the ethanol has been added into WN and WS buffer before first use.

   Keep the cap of the WN and WS bottle tight after each use to avoid volatilization of ethanol. Decreased ethanol content in WN or WS buffer may cause DNA loss during the wash.

5. Wash the column once with 0.5ml of WS Buffer by re-applying the vacuum to draw all the liquid.

Product Contents:

Please check if the contents enclosed match the checklist. Record the date when the first open of each component. It is not recommended to pool together columns or solutions from different lots.

Cat. No.	**EG2001** (50 preps) (amount)	**EG2002** (250 preps) (amount)	Check
GEX Buffer	50 ml	250 ml	☐
WN Buffer	6 ml*	30 ml**	☐
WS Buffer	6 ml*	30 ml**	☐
Elution Buffer	5 ml	25 ml	☐
GP Column	50	250	☐
Collection Tube	50	250	☐
Protocol	1	1	☐

**** For EG2001***

- *Add 24 ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.*

***** For EG2002***

- *Add 120 ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.*

Columns and buffers are available for separated purchase. Please contact us for ordering information.

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 extended periods of time as DNA may be nicked or denatured.*

   - 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 into it.
   - *When agarose percentage of the gel slice is higher than 2 %, adjust the use of GEX Buffer as 5x volumes of the gel slice (100 mg gel with 0.5ml GEX buffer).*

   - Cutting the gel slice into small pieces can facilitate dissolution.
   - If more than 200 mg gel is used in order to harvest more DNA, increase the applied volume of GEX buffer proportionally.

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

   - Ensure that the gel has been completely dissolved before proceeding to step 4. Gently invert the tube, and observe the contents of the vial with back light, to see if there is any gel-like substance remaining.
   - If gel dissolution cannot be completed in 10 minutes, refer to the Troubleshooting Guide on page 12.

4. Place a GP Column onto a Collection Tube. Load no more than 0.7 ml dissolved gel mixture into each column. Centrifuge for 30-60 seconds. Discard the flow-through.

   - If the volume of dissolved mixture is more than 0.7ml, load and filter 0.7ml at each time until all the mixture has been filtrated.

6. Centrifuge the column at full speed for another 3 minutes to remove residual ethanol.
   - *DO NOT remove ethanol by baking the column in an oven, as high temperatures may affect the intactness of the column.*

   - Residual ethanol may affect the quality of DNA and may interfere subsequent enzymatic reactions or sequencing. If necessary, stand the column at room temperature for a few minutes before eluting DNA.

7. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 µl of Elution Buffer (provided) onto the center of the membrane.
   - *Use of elution solution preheated to 60°C can increase the recovery of DNA fragment larger than 5-kb.*

   - 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-centri-fuging 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.

8. Stand the column for 3 minutes and then centrifuge for 1-2 minutes to elute DNA. Store DNA at -20°C.

   - Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30 µl H_2O or buffer, yields more DNA in total than eluting once with 60 µl H_2O or buffer.

Protocol for Spin Method:

1. Pipet 10-100 µl PCR product or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.
   - *Make sure that NO MINERAL OIL is taken. Any mineral oil will adversely interfere with the following protocol.*

   - If more than 100 µl is used in order to harvest more DNA, increase the applied volume of PX buffer proportionally.

2. Insert a GP column onto a Collection Tube. Add all the mixture from step 1 into the column.

   - If the volume of mixture is more than 0.7 ml, load and filter 0.7 ml at each time until all the mixture has been filtrated.

3. Centrifuge for 30-60 seconds. Discard the flow-through.

4. Wash the column once with 0.5 ml WN Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

   - Ensure that the ethanol has been added into WN and WS buffer before first use.
   - Keep the cap of the WN and WS bottle tight after each use to avoid volatilization of ethanol. Decreased ethanol content in WN or WS buffer may cause DNA loss during the wash.

5. Wash the column once with 0.5 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

5. Repeat step 4 for the rest of the mixture.

   - Ensure that the ethanol has been added into WN and WS buffer before first use.
   - Keep the cap of the WN and WS bottle tight after each use to avoid volatilization of ethanol. Decreased ethanol content in WN or WS buffer may cause DNA loss during the wash.

6. Wash the column once with 0.5 ml of WN Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

7. Wash the column once with 0.5 ml of 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.
   - *DO NOT remove ethanol by baking the column in an oven, as high temperatures may affect the intactness of the column.*

   - Residual ethanol may affect the quality of DNA and may interfere subsequent enzymatic reactions or sequencing. If necessary, stand the column at room temperature for a few minutes before eluting DNA.

9. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 µl of Elution Buffer (provided)onto the center of the membrane.
   - *Use of elution solution preheated to 60°C can increase the recovery of DNA fragments larger than 5-kb.*

   - 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.
   - Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30 µl H_2O or buffer, yields more DNA in total than eluting once with 60 µl H_2O or buffer.

10. Stand the column for 3 minutes and then centrifuge at full speed for 1-2 minutes to elute DNA. Store DNA at -20°C.

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 extended periods of time as DNA may be nicked or denatured.*

   - 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.
   - *When agarose percentage of the gel slice is higher than 2 %, adjust the use of GEX Buffer as 5x volumes of the gel slice (100 mg gel with 0.5ml GEX buffer).*

   - Cutting the gel slice into small pieces can facilitate dissolution.
   - If more than 200 mg gel is used to harvest more DNA, increase the applied volume of GEX buffer proportionally.

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

   - Ensure that the gel has been completely dissolved before proceeding to step 4. Gently revert the tube, and observe the contents in vial with back light, to see if there is any gel-like substance remaining.
   - If gel dissolution cannot be completed in 10 minutes, refer to the Troubleshooting Guide on page 12.

4. Insert a GP 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.

   - If the volume of dissolved mixture is more than 0.7ml, load and filter 0.7ml at each time until all the mixture has been filtrated.

Product Contents:

Please check if the contents enclosed match the checklist. Record the date when the first open of each component. It is not recommended to pool together columns or solutions from different lots.

Cat. No.	**PF2001** (50 preps) (amount)	**PF2002** (250 preps) (amount)	Check
PX Buffer	30 ml	150 ml	☐
WN Buffer	6 ml*	30 ml**	☐
WS Buffer	6 ml*	30 ml**	☐
Elution Buffer	0.5 ml	25 ml	☐
GP Column	50	250	☐
Collection Tube	50	250	☐
Protocol	1	1	☐

**** For PF2001***

- *Add 24 ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.*

***** For PF2002***

- *Add 120 ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.*

Columns and buffers are available for separated purchase. Please contact us for ordering information.

PCR *Advanced* PCR Clean Up System

Description

Purification of small-scale DNA using phenol/chloroform extraction and ethanol precipitation is laborious and time-consuming. Viogene **PCR *Advanced*** PCR Clean Up System provides a simple and fast method to purify and clean up PCR products or DNA fragments from components of enzymatic reactions such as enzymes, dNTPs, salts and primers without phenol/chloroform extraction. The system is based on binding of up to 20μg DNA to silica-based membranes in chaotropic salts with average recoveries from 60 to 95% of 100-bp to 10-kb DNA fragments.

Parameter	Value
Average preparation time	5~10 minutes
Workable length of fragment	100-bp ~ 10-kb
Maximal recovery	~95%
Minimal elution volume	15 μl
Maximal capacity	Up to 20 μg
Regular sample volume	10~100 mg

Downstream Applications

- Restriction enzyme digestion
- Modifying enzymatic reaction
- Radioactive and fluorescent labeling
- PCR & qPCR
- Ligation
- Labeling & hybridization
- NGS
- Gene editing

5. Apply the 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 WN Buffer by reapplying vacuum to draw all the liquid.

- Ensure that the ethanol has been added into WN and WS buffer before first use.
- Keep the cap of the WN and WS bottle tight after each use to avoid volatilization of ethanol. Decreased ethanol content in WN or WS buffer may cause DNA loss during the wash.

7. Wash the column once with 0.5 ml of WS Buffer by reapplying the 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.
   - *DO NOT remove ethanol by baking the column in an oven, as high temperatures may affect the intactness of the column.*

- Residual ethanol may affect the quality of DNA and may interfere subsequent enzymatic reactions or sequencing. If necessary, stand the column at room temperature for a few minutes before eluting DNA.

9. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 μl of Elution Buffer (provided)onto the center of the membrane.
   - *Use of elution solution preheated to 60°C can increase the recovery of DNA fragments larger than 5-kb.*

- 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.

10. Stand the column for 3 minutes and then centrifuge for 1-2 minutes to elute DNA. Store DNA at -20°C.

- Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30 μl H_2O or buffer, yields more DNA in total than eluting once with 60 μl H_2O or buffer.

Troubleshooting Guide

Problem	Possible Reason	Solution
Low recovery of DNA fragment	Incomplete dissolution of the gel slice	Check the dissolution mixture with light to see if there is any gel-like substance remained.
	Ineffective DNA elution	DNA elution does not take place well in acidic conditions. Make sure that the ddH_2O used has a pH between 7.0 - 8.5
	Incomplete DNA elution	Complete DNA elution only takes place when elution solution is in full contact with the membrane. Make sure that no less than 15 µl of Elution buffer is dispensed onto the center of 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 increases. Use fresh TAE or TBE buffer each time.
	Overload the column with too much agarose solution	Higher recovery is attained when lower amount of agarose gel is used. Minimize the size of the gel slice by removing extra gel. When gel slice is more than 200 mg, use separated column to proceed the extraction.
	Size of DNA fragment is more than 5-kb	Preheated the elution solution to 60°C before the elution step.
Poor OD_{260}/OD_{280} radio	Use of H_2O of acidic pH to dilute the eluted DNA	Make sure the H_2O has the pH value between 7.0~8.5
Gel slice hard to dissolve	Use high percentage agarose gel	When the agarose percentage is > 2.0%, add GEX Buffer at 5 times the volume of the gel slice (100 mg= 0.5 ml). When the agarose percentage of the gel is > 2.5%, add GEX Buffer at 6 times the volume of the gel slice (100 mg = 0.6 ml). Mix every 1-2 minutes during the incubation until complete dissolution.
	Gel slice is too big (more than 200mg)	Use more than one column for gel slice larger than 200 mg.
Poor performance in downstream applications	Eluted DNA carries salt residue	Wash the column twice with 0.5 ml WS buffer.
	Eluted DNA carries ethanol residue	After washing with WS buffer, discard and centrifuge the column for another 3 minutes. If necessary, stand the column at room temperature for a few minutes before eluting DNA. However, do not remove ethanol by baking the column in an oven as the high temperature may affect the intactness of the column.
	DNA fragment is denatured and becomes single-stranded.	To re-anneal the single-stranded DNA, incubate the tube at 95°C for 2minutes and let it cool down slowly to room temperature. Re-annealed DNA fragments are applicable for all downstream applications.

- If there remains any problem unable to be solved according to the troubleshooting guide, please contact your local distributor for immediate technical support or mailing to us directly at service@viogene.com.