VIOGENE

Cat. No.: PF1001 (50 preps/Kit)
PF1002 (250 preps/Kit)

PCR-M™ Clean Up 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.

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Description

Purification of small-scale DNA using phenol/chloroform extraction and ethanol precipitation is laborious and time-consuming. Viogene PCR-M™ Clean Up System provides a simple and fast method to purify and clean up PCR products or DNA fragments (range from 100 bp to 10 kb) of other enzymatic reactions from enzymes, dNTPs, salts and primers without phenol/chloroform extraction. It is based on the phenomenon of binding of up to 10 μg DNA to silica-based membranes in chaotropic salts with up to 95% recovery.

Preparation Time: 5-10 minutes

Downstream Applications

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

Product Contents

	PF1001 (50 preps) (Cat. No.)	PF1002 (250 preps) (Cat. No.)
PX Buffer	30 ml (PF1001S01)	150 ml (PF1002S01)
WF Buffer	30 ml (001001SWF)	150 ml (001002SWF)
WS Buffer	15 ml* (001001SWS)	45 ml** (001002SWS)
Elution Buffer	5 ml (001001SEB)	25 ml (001002SEB)
PCR-M™ Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces
Protocol	1	1

*For PF1001 (**50 preps**), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

For PF1002 (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 PCR-M™ Clean Up System is stable at 20-25℃ 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℃).

3. For PF1001 (**50** preps), add 60 ml of 98-100% ethanol into WS Buffer bottle **when first open**. For PF1002 (**250** preps), add 180 ml of 98-100% ethanol into WS Buffer bottle **when first open**. Ethanol is provided by the 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_2O, or TE buffer. Since DNA elution only takes place effectively at **pH 7.0 - 8.5**, make sure that the pH of H_2O 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_2O is preferred for elution of DNA immediately used for further enzymatic reactions.

Protocol for Spin Method

1. Pipet 10-100 µl PCR* product (make sure that mineral oil is not taken) or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.

2. Place a PCR-M™ Column onto a 2-ml Collection Tube. Add all the mixture from step 1 into the column.

   Load no more than 0.7 ml mixture into the column each time.

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

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

5. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.

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

6. Centrifuge the column at full speed for another 3 minutes to remove residual ethanol.

   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.

7. Place the column onto a new 1.5-ml centrifuge tube. Add 30-50 µl of Elution Buffer (provided) onto the center of the membrane.

ddH$_2$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.

8. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.

9. Store DNA at -20℃.

* PCR is covered by U.S. patents 4,683,195 and 4,683,202 issued to Hoffmann-La Roche Inc.

Protocol for Vacuum Method

1. Pipet 10-100 µl PCR* product (make sure that mineral oil is not taken) or DNA solution after enzymatic reaction to a new 1.5-ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.

2. Insert a PCR-M™ 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.

   Load no more than 0.7 ml mixture into the column each time.

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

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

5. Wash the column once with 0.7 ml WS Buffer by re-applying vacuum to draw all the liquid.

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

6. Place the column onto a Collection Tube. Centrifuge the column at full speed for 3 minutes to remove residual ethanol.

   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.

7. Place the column onto a new 1.5-ml centrifuge tube. Add 30-50 µl of Elution Buffer (provided) onto the center of the membrane.

ddH$_2$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.

8. Stand the column for 1-2 minutes, and centrifuge for 1-2 minutes to elute DNA.

9. Store DNA at -20℃.

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** Vac-man is a trademark of Promega Corporation.

Troubleshooting Guide

Problem	Possible Reason	Solution
Low recovery of DNA fragment	DNA solution used is more than 100 µl	Divide loading the sample into two or more columns.
		If DNA to be cleaned up is **diluted**, more than 100 µl solution can be used per column. Add 5 µl of more PX Buffer for each 1 µl of extra DNA solution (e.g. add 600 µl PX Buffer to 120 µl DNA solution).
	DNA solution used is of pH less than 7.5	Add 10 µl 0.1 M Tris-HCl (pH 9.0) into the DNA solution before adding PX Buffer.
	Overload the column with too much DNA	Higher recovery is attained from lower amount of loaded DNA. Split loading of high amount of DNA into two or more columns.
	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.

Problem	Possible Reason	Solution
Low recovery of DNA fragment	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 30 µl** of solution is dispensed onto the membrane and is completely absorbed into it before centrifugation.
	Size of DNA product is more than 5 kb	Use elution solution preheated to 60℃.
Poor performance in downstream applications	Eluted DNA carries salt residue	Wash the column twice with 0.7 ml WS Buffer.
	Eluted DNA carries ethanol residue	After wash with WS Buffer, **do** 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 **not** remove ethanol by putting the column into an oven as high temperature may affect the intactness of the column.

Viogene's Hints

1. Milli-Q or double-distilled H_2O stored in a laboratory for a period of time usually becomes acidic due to dissolution of CO_2 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_2O of pH less 7.0 for elution will lead to reduced yield of DNA. Use H_2O of acidic pH (pH 5.0-6.0) to dilute DNA or RNA samples for spectrophotometric analysis will also significantly decrease A_{260}/A_{280} 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 buffer or H_2O, yields more DNA in total than eluting once with 60 µl buffer or H_2O.

3. Use of elution solution preheated to 60℃ can increase recovery of DNA fragment larger than 5 kb.

Reference: Wilfinger, W. W., Mackey, K., and Chomczyski, 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 $1x10^7$ cells	10-45 µg up to 30 µg
Total RNA Midi	GRD1001 GRD1002	10 50	0.1-0.2 g tissue $3-7x10^7$ cells	200-450 µg up to 300 µg
Total RNA Maxi	GRM1001 GRM1002	6 24	0.5-1 g tissue $2-10x10^8$ 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)
VioTaq DNA Polymerase	VT1001	500 U (5 U/µl) 10X PCR Buffer containing 20 mM $MgCl_2$		
VioTwinPack Kit	VTP1001	500 U *VioTaq* DNA Polymerase (5 U/µl) 10X PCR Buffer containing 20 mM $MgCl_2$ 40 mM dNTP mix (10 mM each)		
Clear-band Agarose	AG0050 AG0100	50 g 100 g		