Viogene U.S.A.
P.O.Box 70472 Sunnyvale CA 94086, U.S.A.
e-mail: viogene@hotmail.com
VER.:07A

VIOGENE

User Bulletin

Gel/PCR DNA Isolation System

Purification of small-scale DNA by using phenol/chloroform extraction or ethanol precipitation is laborious and time-consuming. Viogene Gel/PCR DNA Isolation System provides a simple and fast method to extract and isolate DNA fragments (range from 100-bp to 10-kb) from agarose gel or of other enzymatic reactions from enzymes, dNTPs, salts and primers without phenol/chloroform extraction. This system is based on binding of up to 20µg DNA to silica-based membranes in chaotropic salts with average recoveries of 60 to 90 % of 100-bp to 10-kb DNA fragments.

Downstream Application

* Sequancing & PCR
* Restriction digestion & enzymatic reaction
* Ligation
* Labeling & hybridization

Product Contents

Cat. No	GP1001	GP1002
Preps	50	250
GP Buffer	50ml	250ml
WN Buffer	6ml	30ml
WS Buffer	6ml	30ml
Elution Buffer	5ml	25ml
GP Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Gel/PCR DNA Isolation System is shipping and storage at ambient temperature up to 12 months.

If precipitate form by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

❖ **Please read the following notes before starting the procedures.**

- **WARNING**, strong acids and oxidants (like for instance bleach) should not be used together with GP buffer (because this kind of reaction would produce cyanide)!!!

Important Notes

For GP1001

- Add 24 ml of 98 ~ 100 % ethanol to WN Buffer bottle when first open.
- Add 24 ml of 98 ~ 100 % ethanol to WS Buffer bottle when first open.

For GP1002

- Add 120 ml of 98 ~ 100 % ethanol to WN Buffer bottle when first open.
- Add 120 ml of 98 ~ 100 % ethanol to WS Buffer bottle when first open.

- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.

- All procedures should be done at room temperature (20 ~ 25 ℃).
- All centrifugation steps are done at 10,000 x g or 13,000rpm in a microcentrifuge, if not notice.
- 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 (provided) or ddH_2O (pH 7.0 ~ 8.5) is preferred for elution of DNA immediately used for further enzymatic reactions.

A. Gel Extraction Protocol for Spin Method:

1. **Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.**
   Minimize the size of the gel slice by removing extra agarose. Cutting the gel slice into small pieces can facilitate dissolution.

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 GP Buffer into it.**
   When agarose percentage of the gel slice is more than 2 %, add GP Buffer as 5 volumes of the gel slice (100 mg = 0.1 ml).

3. **Incubate at 60 ℃ for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation.**
   Ensure that the gel has been completely dissolved before proceeding to step 4. If the gel slice has not been completely dissolved after 60℃ incubating for 5 to 10 minutes, the gel slice should be too large or more GP Buffer should be added. If DNA size is < 500-bp or > 4.5-kbp, add 0.25 volume of isopropanol of the mixture from Step 3 and mix well. That will increase the recovery of the DNA.

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

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

6. **Wash the column once with 0.5 ml of WN Buffer by centrifuging for 30-60 seconds. Discard the flow-through.**
   Ensure that ethanol has been added into WN Buffer bottle when first open.

7. **Wash the column once with 0.5 ml of 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.

8. **Centrifuge the column at full speed for 3 minutes or more to remove residual ethanol.**
   Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions.

9. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 μl of Elution Buffer onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

10. **Stand the column for 2-3 minutes and centrifuge at full speed⁺ for 1-2 minutes to elute DNA. Store DNA at –20 ℃.**
   ⁺ The full speed depends on the top speed of the microcentrifge.

B. Gel Extraction Protocol for Vacuum Method:

1. **Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.**
   Minimize the size of the gel slice by removing extra agarose. Cutting the gel slice into small pieces can facilitate dissolution.

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 GP Buffer into it.**
   When agarose percentage of the gel slice is more than 2 %, add GP Buffer as 5 volumes of the gel slice (100 mg = 0.1 ml).

3. **Incubate at 60 ℃ for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation.**
   Ensure that the gel has been completely dissolved before proceeding to step 4. If the gel slice has not been completely dissolved after 60℃ incubating for 5 to 10 minutes, the gel slice should be too large or more GP Buffer should be added. If DNA size is < 500-bp or > 4.5-kbp, add 0.25 volume of isopropanol of the mixture from Step 3 and mix well. That will increase the recovery of the DNA.

4. **Insert a GP^{TM} 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. Apply vacuum to draw all the liquid into the manifold.**

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

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

7. **Wash the column once with 0.5ml of 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 or more to remove residual ethanol.**
   Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions.

9. **Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 µl of Elution Buffer onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

10. **Stand the column for 2-3 minutes and centrifuge at full speed^{+} for 1-2 minutes to elute DNA. Store DNA at –20 ℃.**

* Vac-man is a trademark of Promega Inc.

$^{+}$ The full speed depends on the top speed of the microcentrifge.

C. PCR DNA Fragment Isolation 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 GP Buffer and mix well.**
   If DNA size is < 500-bp or > 4.5-kbp, add 0.25 volume of isopropanol of the mixture from Step 1 and mix well. That will increase the recovery of the DNA.

2. **Place a <u>GP™ Column</u> onto a 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 <u>0.5 ml WN Buffer</u> by centrifuging for 30-60 seconds. Discard the flow-through.**
   Ensure that ethanol has been added into WN Buffer bottle when first open.

5. **Wash the column once with <u>0.5 ml WS Buffer</u> 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 <u>at full speed</u> for another 3 minutes or more to remove residual ethanol.**
   It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

7. **Place the column onto a new 1.5 ml centrifuge tube. Add <u>15-30 µl of Elution Buffer</u> (provided) onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

8. **Stand the column for 2-3 minutes and centrifuge <u>at full speed</u>+ for 1-2 minutes to elute DNA.**

9. **Store DNA at 4 ℃ or –20 ℃.**
   + The full speed depends on the top speed of the microcentrifge.

D. PCR DNA fragment Isolation 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 <u>0.5 ml GP Buffer</u> and mix well.**
   If DNA size is < 500-bp or > 4.5-kbp, add 0.25 volume of isopropanol of the mixture from Step 1 and mix well. That will increase the recovery of the DNA.

2. **Insert a <u>GP™ Column</u> into the luer-lock of a vacuum manifold (e.g., Promega's Vac-man**). Add all the mixture from step 1 into the column. Apply the vacuum to pull all the liquid into the manifold.**
3. **Apply vacuum to draw all the liquid into the manifold.**

4. **Wash the column once with <u>0.5 ml WN Buffer</u> by re-applying vacuum to draw all the liquid.**

5. **Wash the column once with <u>0.5 ml WS Buffer</u> by re-applying vacuum to draw all the liquid.**

6. **Place the column onto a Collection Tube. Centrifuge the column <u>at full speed</u> for another 3 minutes or more to remove residual ethanol.**
   It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

7. **Place the column onto a new 1.5 ml centrifuge tube. Add <u>15-30 µl of Elution Buffer</u> (provided) onto the center of the membrane.**
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

8. **Stand the column for 2-3 minutes and centrifuge at full speed+ for 1-2 minutes to elute DNA.**

9. **Store DNA at 4 ℃ or –20 ℃.**

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

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

+ The full speed depends on the top speed of the microcentrifge.