MCLAB
Molecular Cloning Laboratories

Version 2.0

User Manual

Product name: Gel/PCR DNA Isolation System

Cat #: GPAE-100, GPAE-200

Description:

MCLAB's 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 directly from enzymatic reactions to remove dead enzymes, excess dNTPs, salts and primers without phenol/chloroform extraction. This system is based on binding of up to 20μg DNA to silica-based membranes in chaotrophic salts with average recoveries of 60 to 90 % of 100-bp to 10-kb DNA fragments.

Application:

- Sequencing & PCR
- Restriction digestion & enzymatic reaction
- Ligation
- Labeling & hybridization

Contents:

Cat #	GPAE-100	GPAE-200
Preps	50	250
QG Buffer	50ml	250ml
PE Buffer	100ml	500ml
Elution Buffer	5ml	25ml
Column	50	250
Collection Tube	50	250

Shipping & Storage:

MCLAB's Gel/PCR DNA Isolation System is shipped and stored at room temperature (20~25°C).
The shelf life is up to 12 months. If any buffer precipitates, warm up at 37°C to redissolve.

Protocol:

Important Notes

- 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°C).
- 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 0.5ml-1ml QG 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 37°C for 5-10 mins 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 37°C incubating for 5 to 10 minutes, the gel slice might be too large or more QG Buffer should be added. If DNA size is < 500-bp or > 4.5- kbp, add 0.2 volume of isopropanol of the mixture from Step 3 and mix well. That will increase the recovery of the DNA.
4. Place a 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 twice with 1ml of PE Buffer by centrifuging for 30-60 seconds. Discard the flow-through. Ethanol has been added into the PE Buffer.
7. Centrifuge the column at full speed for 5 minutes or more to remove residual ethanol. Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions.
8. 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.
9. Stand the column for 5-10 minutes and centrifuge at full speed for 1-2 minutes to elute DNA. Store DNA at -20°C.

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 0.5-1ml QG 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 37°C 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°C incubating for 5 to 10 minutes, the gel slice should be too large or more QG Buffer should be added. If DNA size is < 500-bp or > 4.5-kbp, add 0.2 volume of isopropanol of the mixture from Step 3 and mix well. That will increase the recovery of the DNA.
4. Insert a column into the luer-lock of a vacuum manifold. 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 twice with PE Buffer by re-applying vacuum to draw all the liquid.
7. Place the column onto a Collection Tube. Centrifuge the column at full speed for 5 minutes or more to remove residual ethanol.
   Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions.
8. 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.

9. Stand the column for 5-10 minutes and centrifuge at full speed+ for 1-2 minutes to elute DNA. Store DNA at -20 °C.

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 0.5-1ml QG Buffer and mix well.
   If DNA size is < 500-bp or > 4.5-kbp, add 0.2 volume of isopropanol of the mixture from Step 1 and mix well. That will increase the recovery of the DNA.
2. Place a column 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 twice with PE Buffer by centrifuging for 30-60 seconds. Discard the flow-through. Ethanol has been added into PE Buffer.
5. Centrifuge the column at full speed for another 5 minutes or more to remove residual ethanol.
   It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.
6. 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.
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.
7. Stand the column for 5-10 minutes and centrifuge at full speed+ for 1-2 minutes to elute DNA.
8. Store DNA at 4°C or -20°C.

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 0.5-1ml QG 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. Insert a column into the luer-lock of a vacuum manifold. 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 twice with PE Buffer by re-applying vacuum to draw all the liquid.
5. Place the column onto a Collection Tube. Centrifuge the column at full speed for another 5 minutes or more to remove residual ethanol.
   It is important to remove ethanol residue, because it may inhibit subsequent enzymatic reactions.
6. Place the column onto a new 1.5 ml centrifuge tube. Add 15-30µl of Elution Buffer (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.
7. Stand the column for 5-10 minutes and centrifuge at full speed for 1-2 minutes to elute the DNA.
8. Store DNA at 4°C or -20°C.