MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0

Product name: Gel Advanced Extraction Miniprep Kit

Cat #: GAE-100, GAE-200

Description:

Gel Advanced Extraction MiniPrep Kit is designed to extract and purify DNA fragments from agarose gel. This kit uses a silica-based membrane in the presence of chemotropic salts to enable the recovery of up to 20 µg of DNA with 95% efficiency. The final eluted DNA is free from agarose, salts and is ready for a wide range of downstream molecular biology applications.

Application:

DNA purified with the Gel Advanced Extraction MiniPrep Kit can be used directly in most molecular biology applications.

- Automated fluorescent and radioactive sequencing & PCR
- Restriction digestion & modifying enzymatic reaction
- Ligation
- Labeling & DNA hybridization

Centrifugation Protocol:

1. Use a clean, sharp scalpel or razor blade to excise the gel containing the DNA fragment of interest.
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 and add 0.5 ml of GEX buffer.
3. Incubate at 60°C for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during the incubation. Stop the incubation when the gel has completely dissolved.
4. Place a 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.
5. Repeat step 4 for the rest of the mixture.
6. Wash the column once with 0.5 ml of PE buffer by centrifuging for 30-60 seconds. Discard the flowthrough.
7. Wash the column once more with 0.5 ml of PE buffer by centrifuging for 30-60 seconds. Discard the flowthrough.
8. Centrifuge the column at full speed (~12,000 rpm) for another 3 minutes to remove residual ethanol.
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.
10. Wait for 3 minutes and then centrifuge at full speed (~12,000 rpm) for 1-2 minutes to elute the DNA. Store the 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.
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.

3. Incubate at 60°C for 5-10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during the incubation. Stop the incubation when the gel has been completely dissolved.
4. Insert a column into the lure-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 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 PE buffer by reapplying the vacuum to draw out all the liquid.
7. Wash the column once more with 0.5 ml of PE buffer by reapplying the vacuum to draw out all the liquid.
8. Place the column onto a collection tube. Centrifuge the column at full speed (12,000 rpm) for 3 minutes to remove the residual ethanol.
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.
10. Wait for 3 minutes and then centrifuge at full speed (12,000 rpm) for 1-2 minutes to elute the DNA. Store the DNA at -20°C.