SiMax™ PCR Products/Agarose Gel Purification

User's Instruction

Cat No.: CSB-50

Description

The SiMax™ PCR Products/Agarose Gel Purification Kit is designed for the rapid purification of PCR products, or for the efficient extraction of DNA fragments from agarose gel. In a high-salt buffer, DNA is bound to the SiMax™ membrane in a spin column. Following a wash step, DNA is eluted in low-salt buffer or water without alcohol precipitation or desalting. This kit removes DNA polymerase, dNTPs, and salts. DNA fragments of 50 bp to 20 kb can be cleaned up within 20 minutes.

Kit Contents

	50 preps
1. NE binding buffer	50 ml
2. Miniprep spin columns with 2 ml collection tubes	50

Principle

The spin column purification kits utilize a silica-gel membrane that selectively absorbs up to 10μg of DNA fragments in the presence of specialized binding buffers. Nucleotides, oligos(<40mer), enzymes, mineral oil and other impurities do not bind to the membrane and will be washed away. The DNA fragments can then be eluted off the column in small volume and used in downstream applications without further processing.

It is highly recommended to equilibrate the spin columns with 3M NaOH prior to use. Just add 200ul 3M NaOH into the columns and centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

A. Protocol for Purification of PCR Products

1. Transfer PCR reaction mixture to a 1.5ml microtube and add 3 volumes of NE Binding Buffer. Invert the tube and mix gently.
2. Transfer the above mixture solution to the Miniprep spin column and let it stand at room temperature for 5 minutes. Centrifuge at 13,000 rpm for 10-20 seconds. Remove the flow-through in the collection tube.

3. Add 500μl of 80% isopropanol (or 80% ethanol) to the column and centrifuge at 13,000 rpm for 10-20 seconds. Remove the flow-through in the collection tube.

4. Repeat washing procedure in step 3. Spin at 13,000 rpm for an additional minute to remove any residual isopropanol (or ethanol).

5. Transfer the column into a clean 1.5ml microtube. Open the lid for 2-3 minutes to let the ethanol volatilize completely. Add 40-50μl of TE Buffer (or ultrapure water). Incubate at room temperature for 3-5 minutes. Centrifuge at 13,000 rpm for 1 minute to elute the DNA.

   Note: It is extremely important to add the TE Buffer into the center part of the column. Incubating the column with TE Buffer at higher temperature (37℃ to 50℃) may slightly increase the yield especially for large (>10,000bp) DNA. Prewarming the TE Buffer at 55℃ to 80℃ may also slightly increase elution efficiency.

6. Store purified DNA at -20℃.

B. B. Protocol for Extraction of DNA fragment from agarose gel

1. Excise the DNA fragment from the agarose gel with a clear, sharp scalpel. Weigh the gel slice (about 100mg) and transfer it to a 1.5 ml microtube.

2. Add 300 μl of NE Binding Buffer to the microtube containing 100 mg gel slice. Incubate at 50-60℃ for 3-5 minutes and invert the tube occasionally until the agarose gel is completely dissolved.

3. Transfer the above mixture solution to the Miniprep spin column and let it stand at room temperature for 5 minutes. Centrifuge at 13,000 rpm for 10-20 seconds. Remove the flow-through in the collection tube.

4. Add 500μl of 80% isopropanol (or 80% ethanol) to the column and centrifuge at 13,000 rpm for 10-20 seconds. Remove the flow-through in the collection tube.

5. Repeat washing procedure in step 4. Spin at 13,000 rpm for an additional minute to remove any residual isopropanol (or ethanol).

6. Transfer the column into a clean 1.5ml microtube. Open the lid for 2-3 minutes to let the ethanol volatilize completely. Add 40-50μl of TE Buffer (or ultrapure water). Incubate at room temperature for 3-5 minutes. Centrifuge at 13,000 rpm for 1 minute to elute the DNA.

   Note: It is extremely important to add the TE Buffer into the center part of the column. Incubating the column with TE Buffer at higher temperature (37℃ to 50℃) may

slightly increase the yield especially for large (>10,000bp) DNA. Prewarming the TE Buffer at 55℃ to 80℃ may also slightly increase elution efficiency.

7. Store purified DNA at -20℃.