Gel & PCR DNA Purification Kit

Catalog Number: EXNA074 Size: 100 Tests

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.



Product Description

The Gel & PCR DNA Purification Kit features a unique adsorption column optimized to recover DNA fragments from both agarose gel extraction and products directly from PCR. The Binding Buffer, included in the kit, provides a visual indication of DNA binding efficiency based on the color change of the pH indicator in the buffer formulation.

The kit features high efficiency and rapid recovery times of DNA fragments between 65 bp to 10 kb. The typical recovery is 70-85% for gel extractions and 90-95% for PCR products. Each adsorption column can bind up to 20 µg of DNA within 10-20 minutes.

The DNA recovered from this kit is suitable for further use in enzyme digestion, PCR, sequencing, library screening, ligation and transcription/translation.

Limitations

• For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Precautions

- · Always wear appropriate protective clothing and follow safe laboratory procedures.
- Minimize the length of UV exposure when excising the band from the gel to avoid damage to DNA.

Materials Provided & Storage

Shipped at ambient temperature. Store the kit at 10-30°C for up to 24 months.

Component	Size	Quantity
Binding Buffer	80 mL	1 Bottle
Wash Buffer	25 mL	1 Bottle
Elution Buffer	6 mL	1 Bottle
Spin Columns	100 Columns	1 Pack
Collection Tubes	100 Tubes	1 Pack

Preparation of Reagents

Wash Buffer: Add 100 mL ethanol (96-100% to the Wash Buffer before use.



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Protocol

Note: If DNA is to be used for sequencing, it is recommended to use ddH_2O elution. Elution Buffer is recommended for longer DNA storage times.

Gel Extraction: Recovery of DNA fragments from agarose gel.

- 1. Using a clean scalpel, cut the target DNA band from the agarose gel and place into a 1.5 mL centrifuge tube. **Note:** *the DNA recovery is improved by minimizing excess gel on the sample. If the gel block is >300 mg, it can be divided into multiple centrifuge tubes.*
- 2. Pipette 500 μL Binding Buffer up and down to mix well. **Note:** *when using 2% agarose gel, add 1000 μL Binding Buffer.*
- 3. Incubate at 55°C for 5 to 10 minutes, shake the vial by inverting multiple times every 2 to 3 minutes until the gel is completely dissolved. Allow the vial to cool to room temperature before the next step. **Note:** *after the gel is completely dissolved, the sample should be yellow. If the sample is purple, add 10 μL 3M sodium acetate solution (pH 5.0). Mix thoroughly until the sample turns yellow.*
- 4. Place the Spin Column into the Collection tube. Mix the sample solution and transfer the sample solution (≤ 800 μL) to the Spin Column. Centrifuge at 11,000 x g for 30 seconds and discard the filtrate. Note: the filtrate can be added back to the Spin Column and repeated once to improve DNA recovery. If the sample solution volume is > 800 μL, repeat step 4 for the remaining solution.
- 5. Add 750 μL Wash Buffer to the Spin Column, centrifuge at 11,000 x g for 30 seconds and discard the filtrate. **Note:** *ensure that 100 mL of ethanol (96-100%) has been added to the Wash Buffer per the Preparation of Reagents.*
- 6. Place the Spin Column back into the Collection Tube, centrifuge at 18,000 x g for 3 minutes and thoroughly remove the residual Wash Buffer. **Note:** *Residual ethanol can affect subsequent enzymatic reactions such as enzyme digestion, PCR, etc.*
- 7. Place the Spin Column in a new 1.5 mL centrifuge tube, add 20-40 μL of Elution Buffer of ddH₂O to the center of the Spin Column membrane, and let stand for 1 minute. Centrifuge at 18,000 x g for 1 minute to elute DNA and store at -20°C. Note: the eluted solution can be pipetted back into the Spin Column, left to stand for 1 minute, and centrifuged at 18,000 x g for 1 minute to increase DNA concentration. Preheating Elution Buffer or ddH₂O to 60°C may improve results.

PCR Product Purification:

- 1. Transfer the PCR product (≤ 100 μL, without mineral oil) into a new 1.5 mL centrifuge tube, add 5 times the volume of Binding Buffer, and vortex to mix. **Note:** *If the PCR product is > 100 μL, divide into multiple tubes.*
- 2. Place the Spin Column into the Collection Tube. Transfer the sample solution into the Spin Column, centrifuge at 11,000 x g for 30 seconds, and discard the filtrate. **Note:** *the filtrate can be added back to the Spin Column and repeated once to improve DNA recovery.*
- 3. Add 750 µL Wash Buffer to the Spin Column, centrifuge at 11,000 x g for 30 seconds and discard the filtrate. **Note:** *ensure that 100 mL of ethanol (96-100%) has been added to the Wash Buffer per the Preparation of Reagents.*
- 4. Place the Spin Column back into the Collection Tube, centrifuge at 18,000 x g for 3 minutes and thoroughly remove the residual Wash Buffer. **Note:** *Residual ethanol can affect subsequent enzymatic reactions such as enzyme digestion, PCR, etc.*
- 5. Place the Spin Column in a new 1.5 mL centrifuge tube, add 20-40 μL of Elution Buffer of ddH₂O to the center of the Spin Column membrane, and let stand for 1 minute. Centrifuge at 18,000 x g for 1 minute to elute DNA and store at -20°C. Note: the eluted solution can be pipetted back into the Spin Column, left to stand for 1 minute, and centrifuged at 18,000 x g for 1 minute to increase DNA concentration. Preheating Elution Buffer or ddH₂O to 60°C may improve results.



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