艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure PCR and Gel DNA Purification Mini Kit

AG 21029
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is designed to provide a quick and simple method for recovering small amounts of DNA fragments. It can be used to recover DNA fragments from PCR products and other enzymatic reaction products, as well as from agarose gels.The product employs a micro purification column with a minimum elution volume as low as 10 µl, and a column binding capacity of up to 12 µg. For samples with low concentration or small quantities, if satisfactory purification results are not achieved using the SteadyPure PCR Purification Kit (Code. AG21003) or the SteadyPure DNA Gel Extraction Kit (Code. AG21005), this product can be used to recover DNA fragments.

The DNA fragments recovered using this product, dissolved in Elution Buffer or sterile water, can be directly used for subsequent gene cloning, DNA sequence analysis, in vitro transcription, restriction enzyme digestion, and various other enzymatic reactions.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure PCR and Gel DNA Purification Mini Kit	AG 21029	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Kit Components	Volume
Buffer MB *1	50 ml
Buffer WB *2	27 ml
Elution Buffer	10 ml
PCR and Gel DNA Mini Columns	50 sets
Collection Tubes	50 pcs

*1: At lower temperatures, Buffer MB may precipitate. Before use, heat the buffer to 37°C until the precipitate dissolves, then proceed with the application.

*2: Before the first use of Buffer WB, add 63 ml of absolute ethanol (with a volume ratio of Buffer WB to absolute ethanol of 3:7). Mix thoroughly and label the bottle. Store at room temperature.

5. Pre-Experimental Notes and Required Experimental Materials

(1) Prepare: absolute ethanol, sterile water, water bath, 1.5 ml centrifuge tubes (RNase-free), pipette tips (RNase-free), and pipettes.

(2) If a precipitate forms in Buffer MB at lower temperatures, heat at 37°C until the precipitate dissolves before use.

(3) Before the first use of Buffer WB, add 63 ml of absolute ethanol (with a volume ratio of Buffer WB to absolute ethanol of 3:7). Mix thoroughly and label the bottle. Store at room temperature.

(4) Preheat the Elution Buffer or sterile water to 50-65°C to improve DNA elution efficiency.

6. Recommendation and Experimental Precautions

(1) This product can be used to recover DNA fragments from PCR products or enzymatic reaction products (see <Procedure - PCR Product Recovery Steps> for details) and from agarose gel (see <Procedure - Gel Recovery Steps> for details).

(2) The maximum binding capacity of the PCR and Gel DNA Mini Columns is 12 μg. It is recommended that the initial sample load does not exceed 12 μg (e.g., if the sample concentration is 200 ng/μl, the initial sample volume should not exceed 60 μl). If the initial sample load exceeds 12 μg, it is advisable to use multiple PCR and Gel DNA Mini Columns for recovery, or use other products from the company: SteadyPure PCR Reaction Purification Kit (Code. AG21003) or SteadyPure DNA Gel Recovery Kit (Code. AG21005).

(3) When cutting the gel, ensure to minimize the amount of gel while cutting out the complete DNA to avoid affecting DNA yield.

(4) Perform the gel cutting quickly to avoid prolonged exposure to UV light, which can cause DNA damage.

(5) Ensure complete dissolution of the gel; incomplete dissolution can lead to DNA loss and insufficient DNA release.

(6) During the procedure, handle the PCR and Gel DNA Mini Columns vertically when placing them into or removing them from the Collection tubes or 1.5 ml centrifuge tubes to avoid contamination from the column tip touching the tube walls.

(7) For steps requiring centrifugation at room temperature, ensure that the centrifuge temperature is maintained at 20-25°C to prevent crystallization in the solution.

(8) For long-term storage of the recovered DNA, it is recommended to elute the DNA using Elution Buffer.

7. Protocol

7.1 Gel Recovery Steps

(1) Add Buffer MB to the PCR product sample or other enzymatic reaction product sample at a volume ratio of 2:1 (Buffer MB to sample), and mix thoroughly (vortexing or repeated pipetting is recommended).
Note: If the initial sample volume is less than 10 µl, supplement with sterile water to a total volume of 10 µl.

(2) Transfer the mixture to the PCR and Gel DNA Mini Columns, let it stand at room temperature for 1 minute, then centrifuge at 12,000 rpm for 1 minute at room temperature. Discard the filtrate.
Note: The initial sample load should not exceed 12 µg (e.g., if the sample concentration is 200 ng/µl, the initial sample volume should not exceed 60 µl). If the initial sample load exceeds 12 µg, it is recommended to use multiple PCR and Gel DNA Mini Columns for recovery or use the SteadyPure PCR Reaction Purification Kit (Code. AG21003).
Note: The maximum capacity of the PCR and Gel DNA Mini Columns is 750 µl. If the liquid volume exceeds this, transfer in multiple steps: load 750 µl of the mixture, let it stand, centrifuge, discard the filtrate, then repeat with the remaining mixture.

(3) Add 750 µl of Buffer WB to the PCR and Gel DNA Mini Columns, centrifuge at 12,000 rpm for 1 minute at room temperature, and discard the filtrate.
Note: Ensure that Buffer WB has the specified volume of anhydrous ethanol added (Buffer WB to anhydrous ethanol ratio is 3:7).

(4) Repeat step 3.

(5) Place the PCR and Gel DNA Mini Columns in new 2.0 ml Collection Tubes and centrifuge at 12,000 rpm for 2 minutes at room temperature.
Note: Handle the columns vertically to avoid contamination from the column tip touching the tube walls.
Note: Placing the columns in new 2.0 ml Collection Tubes helps improve DNA purity.

(6) Place the PCR and Gel DNA Mini Columns in new 1.5 ml centrifuge tubes. Add 10-30 µl of Elution Buffer or sterile water to the center of the membrane, let it stand at room temperature for 1 minute, then centrifuge at 12,000 rpm for 2 minutes at room temperature to elute the DNA. The eluted DNA can be used directly for subsequent analysis or stored at -20°C.
Note: Handle the columns vertically to avoid contamination from the column tip touching the walls of the centrifuge tube.
Note: Heating the Elution Buffer or sterile water to 50-65°C before use can improve DNA elution efficiency.

7.2 PCR Product Recovery Steps

(1) Excise a single target DNA band from the agarose gel and place it in a clean centrifuge tube. Weigh the gel slice to determine its mass.
Note: When excising the gel, remove as much of the gel not containing the target DNA band as possible to minimize the gel mass.
Note: Use a clean paper towel to absorb any electrophoresis buffer on the gel surface.
Note: Tare the centrifuge tube before weighing to obtain the net weight of the gel slice.

(2) Add Buffer MB to the gel slice at a volume ratio of 3:1 (Buffer MB to gel slice) to dissolve the gel slice.
Note: For a 100 mg agarose gel slice, consider its volume as 100 µl, and add 300 µl of Buffer MB accordingly.

(3) Place the mixture containing the gel slice in a 37°C water bath for 5-10 minutes. During the heating process, invert the centrifuge tube every 2 minutes to ensure complete dissolution of the gel slice. After the gel is completely dissolved, let the solution stand until it returns to room temperature. The solution should appear yellow.
Note: If the gel slice is not completely dissolved after 10 minutes in the water bath, extend the dissolution time until it is fully dissolved to avoid DNA loss and incomplete DNA separation due to insufficient dissolution.
Note: If the color of the dissolution solution changes from yellow to orange or pink, add 3 M sodium acetate solution (pH 5.2) in small

increments (10 µl per addition) to the solution, mixing well until the solution returns to yellow.

(4) Transfer the mixture to the PCR and Gel DNA Mini Columns, let it stand at room temperature for 1 minute, then centrifuge at 12,000 rpm for 1 minute at room temperature. Discard the filtrate.
Note: The initial sample load should not exceed 12 µg (e.g., if the sample concentration is 200 ng/µl, the initial sample volume should not exceed 60 µl). If the initial sample load exceeds 12 µg, it is recommended to use multiple PCR and Gel DNA Mini Columns for recovery or use the SteadyPure DNA Gel Recovery Kit (Code. AG21005).
Note: The maximum capacity of the PCR and Gel DNA Mini Columns is 750 µl. If the liquid volume exceeds this, transfer in multiple steps: load 750 µl of the mixture, let it stand, centrifuge, discard the filtrate, then repeat with the remaining mixture.

(5) Add 750 µl of Buffer WB to the PCR and Gel DNA Mini Columns, centrifuge at 12,000 rpm for 1 minute at room temperature, and discard the filtrate.
Note: Ensure that Buffer WB has the specified volume of anhydrous ethanol added (Buffer WB to anhydrous ethanol ratio is 3:7).

(6) Repeat step 5.

(7) Place the PCR and Gel DNA Mini Columns in new 2.0 ml Collection Tubes and centrifuge at 12,000 rpm for 2 minutes at room temperature.
Note: Handle the columns vertically to avoid contamination from the column tip touching the tube walls.
Note: Placing the columns in new 2.0 ml Collection Tubes helps improve DNA purity.

(8) Place the PCR and Gel DNA Mini Columns in new 1.5 ml centrifuge tubes. Add 10-30 µl of Elution Buffer or sterile water to the center of the membrane, let it stand at room temperature for 1 minute, then centrifuge at 12,000 rpm for 2 minutes at room temperature to elute the DNA. The eluted DNA can be used directly for subsequent analysis or stored at -20°C.
Note: Handle the columns vertically to avoid contamination from the column tip touching the walls of the centrifuge tube.
Note: Heating the Elution Buffer or sterile water to 50-65°C before use can improve DNA elution efficiency.

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