



Instruction for Use

***SteadyPure* Agarose Gel DNA Purification Kit**

AG 21005

Version.V1E1

**Research Use Only
Not For Diagnosis Procedures**

1. Description

This Product is optimized for fast, simple and cost effective purification of DNA from agarose gel slices. Retrieved plasmid DNA could be used for further experiments including DNA sequencing analysis, in vitro transcription, transformation, restriction enzyme digestion and other enzymatic reactions.

2. Kit Information

Kit Name	Cat. No	Specification
<i>SteadyPure</i> Agarose Gel DNA Purification Kit	AG 21005	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Kit Components	Volume
Buffer MB	50 ml
Buffer WB*	27 ml
Elution Buffer	10 ml
DNA Fragment Mini Columns	50 sets
Collection Tubes	50 pcs

*: Before use, add 60 ml of 100% ethanol (Ratio of Buffer WB:100% ethanol =3:7) to the Buffer WB bottle. Mix well and properly mark the bottom. Keep at room temperature for storage.

5. Not Provided Experimental Materials

100% Ethanol, 1.5 ml centrifuge tubes (RNase Free), 2.0 ml centrifuge tubes (RNase Free), RNase Free Water, Waterbath Instrument.

6. Recommendation and Experimental Precautions

- (1) For precipitant in Buffer MB, heat and dissolve at 37 °C. Then retain to room temperature before use.
- (2) Large gel slices would affect DNA yield rate negatively. Cut gel slice in small volume. If the gel slice is too large, recommend to increase buffer inputs and repeat column purification steps.
- (3) Recommend to use Elution Buffer for long term storage of retrieved DNA.
- (4) If for downstream DNA sequencing analysis, recommend to use RNase Free Water to elute DNA.
- (5) Operation of gel slicing should be concise and prompt, to avoid long time exposure of DNA under UV light to cause DNA damage.
- (6) Avoid touching the tube wall when adding or relocating solutions. Do not touch the membrane of the tube.
- (7) Adopt adequate protection procedures and gears.

7. Protocol

7.1 Gel Lysis

(1) Add Buffer MB into sliced gel. Volume of Buffer MB should be 3~5 times of the gel solution volume per 100 mg agrose gel slice.

Note: For gel concentration \leq 2%, add 300 μ l of Buffer MB per 100 mg agrose gel slice; For gel concentration \geq 2%, add 500 μ l of Buffer MB per 100 mg agrose gel slice.

Note: When agrose gel input > 500 mg, use multiple Mini Columns for repeated purification operation.

Note: Cut the agrose gel carefully to eliminate portions with no DNA, to reduce gel input for purification.

Note: Use lab towels to cleanse residual electrophoresis solution.

(2) Dissolve gel solution at 37 °C for 5~10 min. During heating dissolution, take out the tube and invert to mix per 2 min. After dissolution, settle and recover to room temperature. The solution shall be visually yellow colored.

Note: Prolong the dissolution time to thoroughly dissolve the gel.

Note: If the solution color is turning from yellow to either orange or pink, add 3 M sodium acetate solution of pH 5.2, mix well, to recover the solution back to yellow colored.

Note: When operating with target DNA fragment of <400 bp, recommend to add isopropanol of final concentration of 20%.

7.2 Relocation to column

Relocate solution from 7.1 to the DNA Fragment Mini Columns. Settle for 1 min at Room Temperature, then Centrifuge for 1 min at 12,000 rpm, Room Temperature. Discard the filtrate at bottom tube.

7.3 Column Purification

(1) Add 750 μ l of Buffer WB to the Plasmid DNA Mini Columns. Centrifuge for 1 min at 12,000 rpm, Room Temperature. Discard the filtrate at bottom tube.

Note: Please ensure that Buffer WB is already added with 100% ethanol at ratio of Buffer RWB:100% ethanol = 3:7.

(2) Repeat Step (1) Once.

(3) Relocate the Mini Columns onto new 2.0 ml Collection tubes. Centrifuge for 2 min at 12,000 rpm, Room Temperature.

Note: Avoid absorption column bottom touching the inner well of bottom tubes, while removing the absorption column.

7.4 DNA Elution and Retrieve

(1) Relocate the Mini Columns onto new 1.5 ml RNase Free Centrifuge tubes. Carefully add 50 μ l of Elution Buffer or RNase Free Water, avoid damaging the membranes. Settle at room temperature for 1 min.

Note: To improve elution efficiency of DNA, recommend to heat the elution buffer or RNase Free Water to 50~65 °C.

(2) Centrifuge for 2 min at 12,000 rpm, Room Temperature to elute the DNA.

(3) Retrieve the DNA at the bottom of centrifuge tubes. Discard the columns.



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