FastPure® Gel DNA Extraction Mini Kit

DC301

Vazyme Biotech Co., Ltd.
Web: www.vazyme.com
Tel: 400-600-9335
Sales: global@vazyme.com
Support: global@vazyme.com
Service: global@vazyme.com

Instruction for Use

Version 21.1

www.vazyme.com

Contents

01/Product Description

This Kit uses an optimized buffer system and silica gel column purification technology to recover DNA fragments of 70 bp - 20 kb from TAE/TBE agarose gels of various concentrations, which can be sol transferred to a DNA adsorption column and directly centrifuged under high salt conditions to specifically adsorb DNA and remove other impurities. In addition, the kit can directly purify DNA fragments from PCR products, enzymatic reaction systems or crude DNA products obtained by other methods, and remove impurities such as proteins, other organic compounds, inorganic salt ions and oligonucleotide primers. It can ensure that the purification can be completed within 10 - 15 min. The purified DNA can be used directly for ligation, transformation, enzymatic digestion, in vitro transcription, PCR, sequencing, microinjection, and other molecular studies.

02/Components

Components	DC301-01 (100 rxns)
Buffer GDP	80 ml
Buffer GW	2 × 20 ml
Elution Buffer	20 ml
FastPure DNA Mini Columns-G	100
Collection Tubes 2 ml	100

Buffer GDP: DNA binding buffer.

Buffer GW: Washing buffer; add absolute ethanol by the indicated volume on the bottle before use.

Elution Buffer: Elution.

FastPure DNA Mini Columns-G: DNA filtration columns.

Collection Tubes 2 ml: Collection tubes for filtrate.

03/Storage

Store at 15 ~ 25℃, transport at room temperature.

04/Applications

Capable for purifying 70 bp - 20 kb DNA fragments from TAE/TBE agarose gel in different concentration; suitable for DNA from PCR, enzymatic reaction system and other methods.

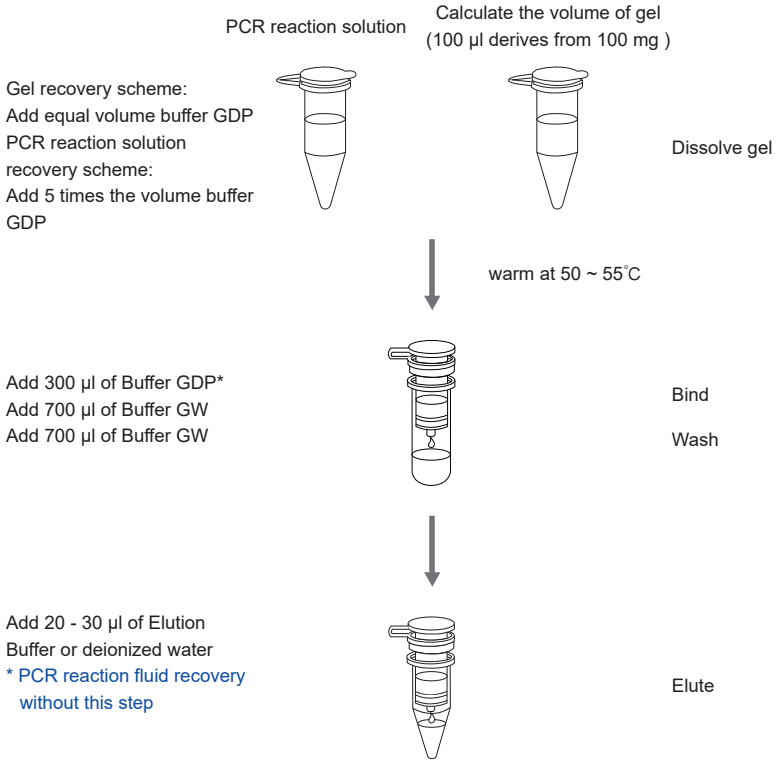
05/Self-prepared Materials

1.5 ml sterilized tubes, 100% ethanol and isopropanol (when DNA fragment ≤100 bp, add 1 volume isopropanol, equal to gel slice), water bath.

06/Notes

1. Add 80 ml of ethanol to dilute Buffer GW as indicated on tag prior to use, store at room temperature.
2. If the buffer GDP is easy to precipitate during low-temperature storage, it can be placed at room temperature for a period of time before use. If necessary, it can be preheated in a 37˚C water bath until the precipitate is completely dissolved, and then it can be used after mixing.
3. Set the water bath temperature to 50 ~ 55˚C in advance.
4. In step 1, minimizing the size of gel slice will significantly reduce the dissolving time and increases recovery efficacy (Linearized DNA is easily to hydrolyze when continually exposed at high temperature). Do not expose DNA gel to UV for long time, as ultraviolet light can cause DNA damage.
5. Dissolve the gel in step 2 completely, otherwise the DNA recovery efficacy will be seriously affected.
6. Warm Eluent Buffer or deionized water to 55˚C , which is helpful to improve DNA elution efficacy. It is recommended to store DNA in eluent of 2.5 mM Tris-HCl, pH 7.0 - 8.5.

07/Mechanism & Workflow



08/Experiment Process

Add 80 ml of ethanol to dilute Buffer GW as indicated on tag before use, store at room temperature.

08-1/Gel recovery program

1. After DNA electrophoresis for fractionating DNA fragments, excise the single stripe of DNA fragment from the agarose gel under UV light. It is recommended to use absorbent paper to absorb apparent moisture of gel and minimize the size of the gel slice by removing extra agarose as possible as you can. Weigh the gel slice (without microcentrifuge tube) to calculate its volume: The volume of 100 mg gel slice is approximately 100 µl, assuming the density is 1 g/ml.
2. Add 1 volume Buffer GDP, incubate at 50 ~ 55˚C for 7 - 10 min (according to the gel size, adjust incubation time until the gel completely dissolved). Invert the tube 2 times during the incubation.
   ▲ Addition of 1 - 3 volume of Buffer GDP will not influence DNA recovery efficacy. If the DNA fragment to be recovered <100 bp, 3 volumes of Buffer GDP need to be added; when the gel slice has dissolved completely, add 1 volume of isopropanol and mix thoroughly, then continue to the next step.
3. Spin briefly to bring the sample to the bottom of the tube, insert a FastPure DNA Mini Columns-G into a 2 ml Collection Tube, carefully transfer the solution maximally of 700 µl once a time to the filtration columns, centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec.
4. Discard the filtrate and reuse the Collection Tube, add 300 µl of Buffer GDP to the column, incubate at room temperature for 1 min, centrifuge at 12,000 rpm (13,400 × g) for 30 - 60 sec.
5. Discard the filtrate and reuse the Collection Tube, add 700 µl of Buffer GW (with ethanol added) to the filtration column, centrifuge at 12,000 rpm (13,400 × g) gfor 30 - 60 sec.
   ▲ Please add Buffer GW around the adsorption column wall, or add Buffer GW back cover and mix it upside down for 2 - 3 times to help completely flush the salt adhering to the pipe wall.
6. Repeat step 5.
   ▲ flushing with Buffer GW twice can ensure that the salt is completely removed and eliminate the impact on subsequent experiments.
7. Discard the filtrate and reuse the Collection Tube, centrifuge the empty column at 12,000 rpm (13,400 × g) for 2 min.
8. Insert the column into a clean 1.5 ml microcentrifuge tube, add 20 - 30 µl of Elution Buffer to the center of the column membrane, incubate for 2 min, and then centrifuge at 12,000 rpm (13,400 × g) for 1 min. Discard the filtration column, store DNA at -20˚C.
   ▲ transferring the supernatant of Step 8 to the filtration column to elute again and preheating the elution buffer to 55˚C (when DNA fragment >3 kb) may be helpful to increase the recovery efficacy.

08-2/PCR reaction solution recovery scheme

This protocol is applicable to purify DNA fragments from PCR products, enzymatic reaction system and other DNA crude products (including genetic DNA), effectively removing nucleotides, primers, dimers, salts, enzymes and other impurities.

1. Briefly spin PCR products, enzymatic reaction solution, and other DNA crude products. Estimate their volume with pipette and transfer to a sterilized 1.5 ml or 2 ml tube. Add ddH_2O until the volume up to 100 μl; while for genomic DNA with high concentration , diluting to 300 μl with ddH_2O will help to improve recovery efficacy.
2. Add 5 volume of Buffer GDP, mix thoroughly by inverting or vortexing. If DNA fragment of interest ≤100 bp, additional 1.5 volumes (samples + Buffer GDP) of ethanol need to be added.
3. Insert the column back into the Collection Tube, transfer the mix to the column, centrifuge at 10,000 rpm (8,000 × g) for 30 - 60 sec. If the volume of mixed liquid is >700 μl, place the adsorption column in the recovery header, and transfer the remaining solution to the adsorption column at 12,000 rpm (13,400 × g) Centrifuge for 30 - 60 sec.
4. The next performance refers to the Step 5 - 8 of protocol 08-1.

09/FAQ & Troubleshooting

◇ Low DNA recovery efficacy

The agarose gel dissolved incompletely: Remove extra agarose as possible as you can and invert the tube during the incubation to make sure the gel slice melted completely.

The short DNA fragment : If the DNA fragment ≤100 bp, add 1 volume of isopropanol.

Regent incorrectly prepared: Add correct volume of ethanol to Buffer GW to keep the final ethanol concentration within 80%.

Low elution efficacy: Warm the Elution Buffer to 55°C and elute twice.

◇ Unsatisfactory downstream result

Salt pollution: Ensure that wash the pellet with Buffer GW twice,In addition, Buffer WB is added around the tube wall of the adsorption column, or the back cover is added and mixed upside down for 2 - 3 times to help completely flush the salt adhering to the tube wall.

Residual agarose gel: Remove extra agarose as possible as you can and invert the tube during the incubation to make a complete melting.

The ssDNA contained in eluate: Incubate the eluate at 95℃ for 2 min, and cool the tube slowly to room temperature to make ssDNA anneal again.