FastPure Gel DNA Extraction Mini Kit

DC301

Instruction for Use

Version 22.1

Contents

01/Product Description

This kit adopts optimized buffer system and silica gel column purification technology, which can recover 70 bp - 20 kb DNA fragments from various concentrations of TAE or TBE agarose gel. DNA adsorption column can specially adsorp DNA under high-salt condition. 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, enzyme digestion, in vitro transcription, PCR, sequencing, microinjection, etc.

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 adsorption columns.

Collection Tubes 2 ml: Collection tubes for filtrate.

03/Storage

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

04/Applications

Various concentrations of TAE or TBE agarose gel; PCR products, enzymatic reaction systems or other crude DNA products obtained by various methods. Recovered fragments ranged from 70 bp - 20 kb.

05/Self-prepared Materials

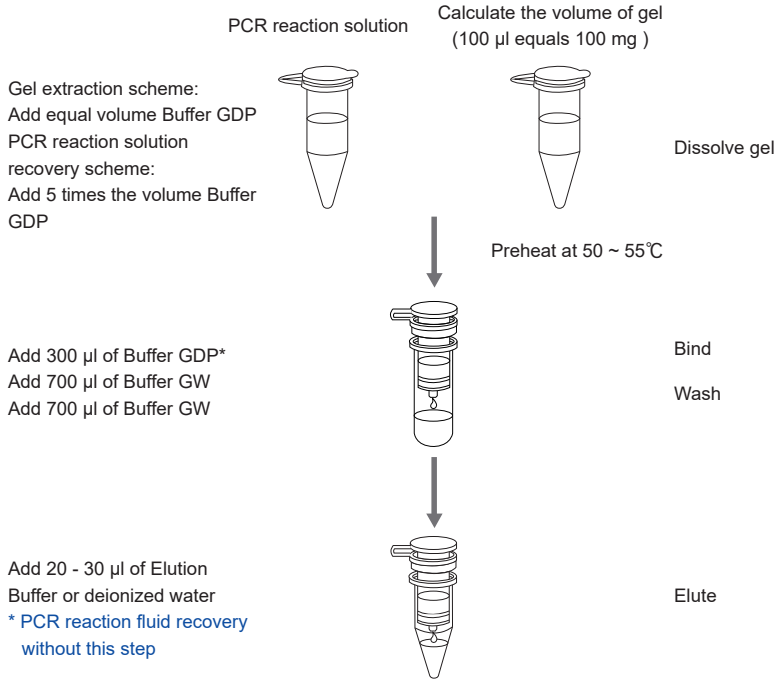
1.5 ml sterilized tubes, absolute ethanol and isopropanol (when DNA fragment ≤100 bp, add 1 volume isopropanol to 1 volume gel), water bath.

06/Notes

For research use only. Not for use in diagnostic procedures.

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℃ 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℃ in advance.
4. In 08-1/Gel extraction program step 1, minimizing the size of gel slice will significantly reduce the dissolving time and increases recovery efficiency (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 08-1/Gel extraction program step 2 completely, otherwise the DNA recovery efficiency will be seriously affected.
6. Preheat Elution Buffer or ddH_2O to 55℃, which is helpful to improve DNA elution efficiency. 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 extraction 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 equal volume Buffer GDP, incubate at 50 ~ 55℃ 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 volumes of Buffer GDP will not influence DNA recovery efficiency. 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. Centrifuge briefly to bring the sample to the bottom of the tube, insert the FastPure DNA Mini Columns-G into the Collection Tubes 2 ml, carefully transfer the solution maximally of 700 µl once a time to the filtration columns, centrifuge at 12,000 rpm (13,800 × g) for 30 - 60 sec.
4. Discard the filtrate and add 300 µl of Buffer GDP to the column, incubate at room temperature for 1 min, centrifuge at 12,000 rpm (13,800 × g) for 30 - 60 sec.
5. Discard the filtrate and add 700 µl of Buffer GW (check if absolute ethanol has been added in advance!) to the column, centrifuge at 12,000 rpm (13,800 × g) for 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 tube 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 centrifuge the empty column at 12,000 rpm (13,800 × 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 (13,800 × g) for 1 min. Discard the column, store the obtained DNA at -20℃.
   ▲ Transferring the supernatant of step 8 to the column to elute again and preheating the Elution Buffer to 55℃ (when DNA fragment >3 kb) may be helpful to increase the recovery efficiency.

08-2/PCR products recovery program

This protocol is applicable to purify DNA fragments from PCR products, enzymatic reaction system and other DNA crude products (including genetic DNA). This solution can efficiently remove various nucleotides, primers, primer dimers, salt molecules, enzymes and other impurities.

1. Briefly centrifuge 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 efficiency.
2. Add 5 volumes 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 mixtrue to the column, centrifuge at 12,000 rpm (13,800 × g) for 30 - 60 sec. If the volume of the mixed solution is >700 µl, put the adsorption column back into the collection tube, transfer the remaining solution to the adsorption column, and centrifuge at 12,000 rpm (13,800 × g) for 30 - 60 sec.
4. The next performance refers to the step 5 - 8 of 08-1/Gel extraction program.

09/FAQ & Troubleshooting

◇ Low DNA recovery

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 equal volume of isopropanol.
Regent incorrectly prepared: Add correct volume of ethanol to Buffer GW to keep the final ethanol concentration within 80%.
Low elution efficiency: Preheat the Elution Buffer to 55℃ and elute twice.

◇ Unsatisfactory downstream result

Salt pollution: Make sure to use Buffer GW to wash twice; in addition, add Buffer GW along the wall of the adsorption column, or add Buffer GW and then fasten the cap of the tube and mix it upside down for 2 - 3 times to help completely wash away the salt attached 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 product: Incubate the product at 95℃ for 2 min, and cool the tube slowly to room temperature to make ssDNA anneal again.

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