FastPure Gel DNA Extraction Mini Kit

Catalog # DC301

Version 5.1 Vazyme biotech co., ltd.

1 Introduction

This Kit with optimized buffer and silicon purification column is able to purify 70 bp - 20 kb DNA fragments from TAE / TBE agarose gel in various concentrations. Dissolve the gel and transfer the lysate to DNA filtration columns, centrifuge directly at high-salt condition to make a specific binding of DNA and the removal of impurities, totally within 10 - 15 min to finish the purifying. Besides, the kit can purify DNA fragments directly from PCR products, enzymatic reaction system, and crude DNA products, etc., efficiently removing proteins, other organic compounds, salts, nucleotides primers, etc. The purified DNA can be directly used for molecular biology research, such as ligation, transformation, restriction enzyme digestion, transcription in vitro, PCR, sequencing, microinjection, etc.

2 Contents

Components	DC301-01 (100 rxn)
Buffer GDP	80 ml
Buffer GW	2 x 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 moderate ethanol to dilute Buffer PW2 as indicated on tag prior to use.
Elution Buffer: Elution.
FastPure DNA Mini Columns-G: DNA filtration columns.
Collection Tubes 2 ml: Collection tubes for filtrate.

3 Storage

All regents should be stored at room temperature (15℃- 25℃) .

▲The buffer forms precipitate easily when stored at low temperature. Dissolve at room temperature for a while or at 37℃ for 10 min if necessary to thaw the precipitate and mixed thoroughly before use.

4 Application

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.

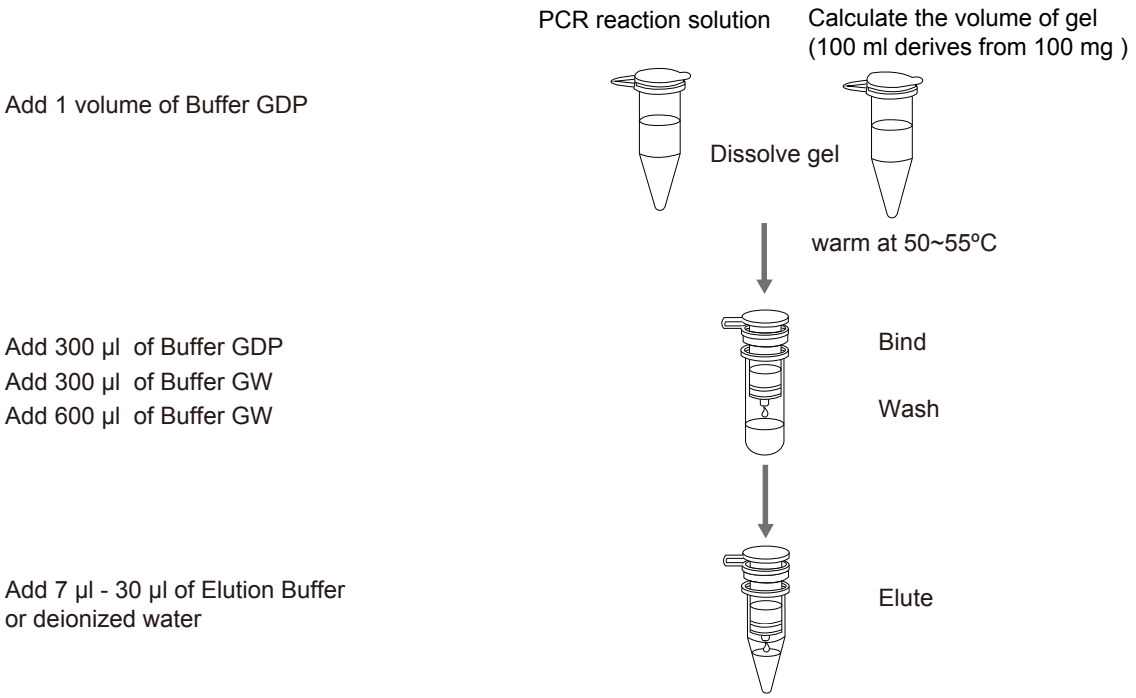
5 Additional Materials Required

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

6 Tips

- Add 80 ml of ethanol to dilute Buffer GW as indicated on tag prior to use, store at room temperature.
- Prewarm the water bath to 50℃- 55℃ .
- 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.
- Dissolve the gel in step2 completely, otherwise the DNA recovery efficacy will be seriously affected.
- Warm Eluent Buffer or deionized water to 55℃ , 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.

7 Principle and Procedure



8 Protocol

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

8.1 DNA Purification from Agarose Gel

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℃ 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 x 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 x g for 30 - 60 sec.

5. Discard the filtrate and reuse the Collection Tube, add 600 µl of Buffer GW (with ethanol added) to the filtration column, centrifuge at 12,000 x g for 30 - 60 sec.
6. Repeat step 5.
7. Discard the filtrate and reuse the Collection Tube, centrifuge the empty column at 12,000 x g for 2 min.
8. Insert the column into a clean 1.5 ml microcentrifuge tube, add 7 µl - 30 µl of Elution Buffer to the center of the column membrane, incubate for 2 min, and then centrifuge at 12,000 x g for 1min. Discard the filtration column, store DNA at -20℃ .
   ▲transferring the supernatant of **Step8** to the filtration column to elute again and preheating the elution buffer to 55°C (when DNA fragment >3kb) may be helpful to increase the recovery efficacy.

8.2 DNA Purification from PCR Reaction Solution

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/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 1 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 x g for 30 - 60 sec. The next performance refers to the **Step 5 - 8 of protocol 08-1**.

9 Troubleshooting

1. 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 PW2 to keep the final ethanol concentration within 80%.
   Low elution efficacy: Warm the elution buffer to 55°C and elute twice.
2. Unsatisfactory downstream result
   Salt pollution: Ensure that wash the pellet with Buffer PW2 twice.
   Residual agarose gel: Remove extra agarose as possible as you can and invert the tube during the incubation to make a complete melting.
   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.