TIANGEN®

Universal DNA Purification Kit

For purification of DNA fragments from agarose gels and solutions

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Universal DNA Purification Kit

(Spin Column)

Cat. no. GDP214

Kit Contents

Contents	GDP214-02 50 preps	GDP214-03 200 preps
Buffer PC	25 ml	100 ml
Buffer BL	30 ml	120 ml
Buffer PW	15 ml	50 ml
Buffer EB	15 ml	30 ml
Spin Columns CB2	50	200
Collection Tubes 2 ml	50	200
Gel Cutter	5	20
Handbook	1	1

Storage

Universal DNA Purification Kit should be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use.

Introduction

Universal DNA Purification Kit provides a fast, simple, and high-effective DNA purification method from TAE/TBE agarose gel and from PCR reactions. Buffer PC contains a pH indicator, allowing easy determination of the optimal pH for DNA binding. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using Spin Column CB2. The recovery efficiency is between 60%-80%. For the length of DNA fragment is longer than 10 kb, it is recommended to use TIANgel Mini/Midi Purification Kit (Cat. no. GDP219) to purify DNA from agarose gel or use TIANquick Mini/Midi Purification Kit (Cat. no. GDP204) to purify DNA from solution.

The DNA purified by this kit is high quality and serves as an excellent template for restriction enzyme digestion, PCR analysis, sequencing, Genomic DNA library, DNA ligation and transformation procedures.

Important Notes Before Starting

1. Buffer BL can improve the absorption capability and stability of the silica membrane, and eliminate any negative influence from environmental factors such as high temperature or humidness. Check buffers before use for salt precipitation. Redissolve any precipitate by incubating at 37°C for 10 minutes.
2. DNA adsorption requires pH ≤7.5, and the pH indicator in the Buffer PC will appear yellow in this range.

Protocol

Ensure that ethanol (96-100%) has been added to Buffer PW as indicated on tag before use.

Purification of DNA fragment from the agarose gel

1. Column equilibration: Add 500 µl Buffer BL to the Spin Column CB2 **(in a 2 ml Collection Tube)**. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through, and set the Spin Column CB2 back into the Collection Tube **(Please use fresh treated spin columns)**.
2. Cut the target DNA band from the agarose gel (remove the extra part as much as possible) and transfer it into a clean centrifuge tube, and then weigh the gel block.

Note: When use the Gel Cutter for cutting the gel, point the mouth of the Gel Cutter at the target DNA band in the agarose gel and press to cut. After cutting the gel, push the center rod to push the gel block into the clean centrifugal tube. Single cutting and continuous cutting can be carried out according to the width of the gel well.

3. Add equal volume of Buffer PC to the volume of gel (For estimation purpose, 100 mg gel is approximately 100 µl Buffer PC. Use the Gel Cutter to cut the target DNA band from a 1% agarose gel, and the single gel block is about 0.06 g. The weight of the actual gel block is related to the gel concentration and thickness). Incubate at 50°C for about 10 min by inverting up and down the tube until the agarose gel dissolved completely **(If the agarose gel is too large, please cut the agarose gel into several pieces firstly)**.

   Note: If the DNA fragment <150 bp, add 3× volumes of Buffer PC to agarose gel sample; please load to column after the solution turns to be room temperature, because column binds DNA stronger at room temperature. After the agarose gel dissolved completely if the solution turns to be yellow, continue the next steps. If the solution is orange or violet, add 10 µl 3 M CH_3COONa (pH 5.0) to the solution until the solution becomes yellow and then continue. (Buffer PC contains a pH indicator and become yellow when pH≤7.5, allowing easy determination of the optimal pH for DNA binding.)

4. Transfer the whole solution to the Spin Column CB2 **(in a 2 ml Collection Tube)**. Centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through and set the Spin Column CB2 back into the Collection Tube.
5. Add 600 µl Buffer PW **(Ensure that ethanol (96-100%) has been added to Buffer PW before use)** into the Spin Column CB2 and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through, and set the Spin Column CB2 back to the Collection Tube.

   Note: If the DNA will be used for salt-sensitive applications, such as blunted end ligation and direct sequencing, allow the column to stand 2-5 min after addition of Buffer PW and before centrifuge to remove salt contamination completely.

6. Repeat Step 5.
7. Set the Spin Column CB2 back to the Collection Tube and centrifuge the column at 12,000 rpm (~13,400 × g) for an additional 2 min to remove residual wash buffer. Discard the flow-through, and allow the column to

air dry with the cap open for 2-5 mins to dry the membrane.

Note: Residual ethanol from Buffer PW will influence the subsequent enzymatic and sequencing reactions.

8. To elute DNA, place the column in a clean 1.5 ml microcentrifuge tube. **Add appropriate volume of Buffer EB (For fragments >4 kb, Buffer EB should be preheated to 65-70°C) to the center of the membrane,** incubate at room temperature (15-30°C) for 2 min, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

   Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. The pH value of elution buffer will have some influence in eluting. Buffer EB or distilled water (pH 7.0-8.5) is suggested to elute plasmid DNA. For long-term storage of DNA, eluting in Buffer EB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis. Repeat step 8 to increase plasmid recovery efficiency.

Purification of DNA from PCR reaction Mix and enzymatic reaction Mix

1. Column equilibration: add 500 µl Buffer BL to the Spin Column CB2 (In a 2 ml Collection Tube). Centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through, then set Spin Column CB2 back into the Collection Tube **(Please use fresh treated spin columns)**.
2. Estimate the volume of PCR reaction mix or enzymatic reaction mix. Add equal volume of Buffer PC to the PCR reaction mix and mix gently (It is not necessary to remove mineral oil or kerosene).

   Note: If the DNA fragment < 150 bp, add 3× volumes of Buffer PC to the PCR reaction mix and mix the solution. If it turns to be yellow, continue next steps. If the solution is orange or violet, add 10 µl 3 M CH_3COONa (pH 5.0) to the solution until the solution becomes yellow and continue next steps.
3. Transfer the whole solution to the Spin Column CB2 (in a 2 ml Collection Tube). Incubate at room temperature (15-30°C) for 2 min and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through and set the Spin Column CB2 back into the Collection Tube.

   Note: The volume of the Spin Column CB2 is 800 µl. If the volume of the whole solution is more than 800 µl, it is recommended to add the

solution to the Spin Column CB2 several times.

4. Add 600 µl Buffer PW **(Ensure that ethanol (96-100%) has been added to Buffer PW before use)** into the Spin Column CB2 and centrifuge at 12,000 rpm (~13,400 × g) for 1 min. Discard the flow-through, and set the Spin Column CB2 back to the Collection Tube.

   Note: If the DNA will be used for salt-sensitive applications, such as blunted end ligation and direct sequencing, allow the column to stand 2-5 min after addition of Buffer PW and before centrifuge to remove salt contamination completely.

5. Repeat Step 4.

6. Set the Spin Column CB2 back to the Collection Tube and centrifuge the column for an additional 2 min to remove residual wash buffer. Discard the flow-through, and allow the column to air dry with the cap open for 2-5 minutes to dry the membrane.

   Note: Residual ethanol from Buffer PW will influence the subsequent enzymatic and sequencing reactions.

7. To elute DNA, place the column CB2 in a clean 1.5 ml microcentrifuge tube. **Add appropriate volume of Buffer EB (For fragments >4 kb, Buffer EB should be preheated to 65-70°C) to the center of the membrane,** incubate at room temperature (15-30°C) for 2 min, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

   Note: If the volume of elution buffer is less than 30 µl, it may affect recovery efficiency. The pH value of elution buffer will have some influence in eluting. Buffer EB or distilled water (pH 7.0-8.5) is suggested to elute plasmid DNA. For long-term storage of DNA, eluting in Buffer EB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis. Repeat step 7 to increase plasmid recovery efficiency.