TOOLS Gel extraction and PCR cleanup Kit

To recover or purify DNA fragments from agarose gels/ PCR reactions

TOOLS Gel extraction and PCR cleanup Kit (Cat no. ST-G01)

Kit contents

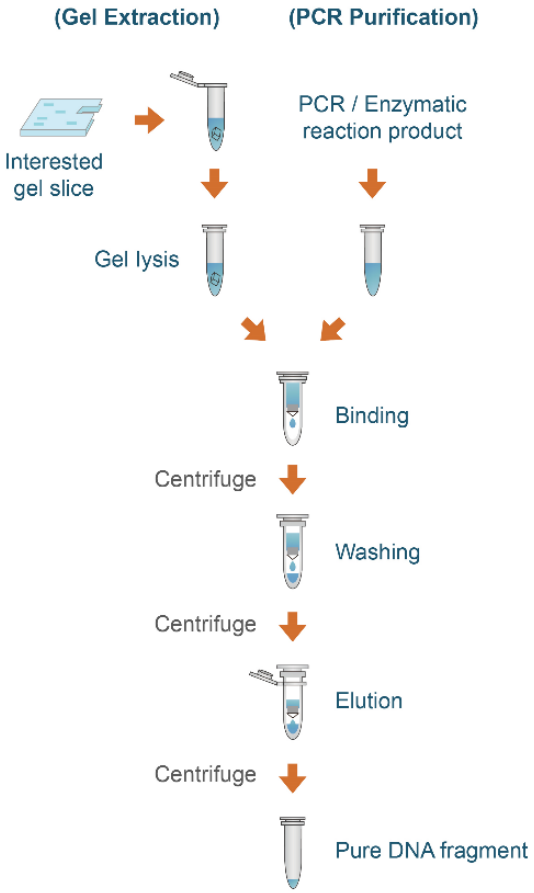
Component	ST-G01 (300 preps)
DS Buffer	240 mL
Wash Buffer* (Add Ethanol), 2 bottles	50 mL x 2 bottles (200 mL)
EB Buffer	30 mL
DF Columns	300 tubes
2 mL Collection Tubes	300 tubes

*Add 200 mL absolute ethanol into Wash Buffer before initial use.

Storage

Gel extraction and PCR cleanup Kit can be stored dry at room temperature (15-25°C) for up to 12 months. The kit can be stored for up to 12 months without showing any reduction in performance and quality

Workflow



Introduction

Gel extraction and PCR cleanup Kit provides a fast, simple, and high-effective DNA purification from agarose gel and from PCR reactions. The unique Spin Column is able to retrieve DNA fragments from TAE or TBE. It is suitable for PCR clean-up and gel filtration. The recovery efficiency is more than 80%. 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 Note

For the first time using, please add 200 ml absolute ethanol to Wash Buffer as the instruction.

Sample: up to 300 mg of agarose gel and up to 100 µl of reaction solution

Recovery: 70% ~ 90% for Gel extraction and 90% ~ 95% for PCR clean-up

DNA Binding capacity of spin column: 20 µg

Sample size: up to 300 mg of agarose gel and up to 100 µl of reaction solution

DNA size: 65 bp ~ 10 kbp

Protocol

A. *Purify DNA fragment from agarose gel*

*Ensure the absolute ethanol was add into Wash Buffer before initial use.

1. Cut the single DNA band from the agarose gel. Weigh the agarose gel slice in a clean Falcon tube.

Note: The maximum weight of the gel slice is 300 mg.

2. Add **500 µl** of DS Buffer to the sample and mix by vortexing. (For > 2% agarose gels, add **1000 µl** of DS Buffer). Incubate at 55~60℃ water bath for 10-15 minutes and vortex the tube every 2-3 min until the gel slice dissolved completely. During incubation, interval vortex can accelerate the gel dissolved. Make sure the gel slice has been dissolved completely before proceed the next step.

Note：After the gel dissolves within DS Buffer, please wait until buffer cools down for 2 minutes, and move it to a DF column.

3. Place **DF column** in a **2 ml Collection Tube** and transfer the whole solution to DF Column (~**800 µl**). Centrifuge 1 minute at 13,000 rpm (17,900 x g) and discard the flow through. If the sample mixture is more than 800 µl, repeat this step for the rest sample mixture. Discard the supernatant completely. And put the DF Column back to the Collection Tube.

4. Wash the DF Column by **750 µl Wash Buffer** and stand for 1 minute at room temperature. Centrifuge 1 minute at 13,000 rpm (17,900 x g). Discard the supernatant completely, and put the DF Column back to the Collection Tube.

5. Repeat **step 4** again.

6. Centrifuge it for an **additional 3 minutes at** 13,000 rpm (17,900 x g) to remove remaining Wash Buffer.

Note: Residual Wash Buffer will affect subsequent experiments.

7. Place the DF Column into a clean 1.5 ml tube. To elute DNA, and add **20~50µl EB Buffer** on the center of the DF Column. Then stand for at least 2 minutes, and centrifuge 2 minutes at 13,000 rpm (17,900 x g) to collect the DNA solution.

Note:

- **In order to increase the retrieve quantity, take the centrifuged DNA solution back to the DF column and centrifuge again.**
- **For a more effective elution, make sure that the EB Buffer is dispensed onto the membrane center and is absorbed completely.**
- **If the eluting DNA fragments >5kb, using the pre-heated EB Buffer (55~60℃) or incubating the tubes at 55~60℃ for 3 mins will increase the DNA yield.**
- **The recovery efficiency will decrease if the pH is not at the range of pH 7.0 to 8.5. And the product should be stored at -20℃ to avoid DNA degrading.**

B. ***Purify double-stranded DNA fragment from solution (PCR reactions, and enzymatic reaction, etc.).***

*Ensure the absolute ethanol was add into Wash Buffer before initial use.

1. Add **5 times volume** of DS Buffer into the PCR reaction solution and mix it. The maximum volume of PCR product is **100 µl** (excluding oil). Do not excess this limit. If PCR product is more than 100 µl, separate it into multiple tubes.

2. Place **DF column** in a **2 ml Collection Tube** and transfer the whole solution to DF Column. Centrifuge 1 minute at 13,000 rpm (17,900 x g). Discard the supernatant completely. And place the DF Column into a Collection Tube.

3. Wash the DF Column by **750 µl Wash Buffer** and stand for 1 minute at room temperature. Centrifuge 1 minute at 13,000 rpm (17,900 x g). Discard the supernatant completely, and put the DF Column back to the Collection Tube.

4. Repeat **step 3** again.

5. Centrifuge it for an **additional 3 minutes at** 13,000 rpm (17,900 x g) to remove remaining Wash Buffer.

Note: Residual Wash Buffer will affect subsequent experiments.

6. Place the DF Column into a clean 1.5 ml tube. To elute DNA, and add **20~40µl EB Buffer** on the center of the DF Column. Then stand for at least 2 minutes, and centrifuge 2 minutes at 13,000 rpm (17,900 x g) to collect the DNA solution.

Note:

- **In order to increase the retrieve quantity, take the centrifuged DNA solution back to the DF column and centrifuge again.**
- **For a more effective elution, make sure that the EB Buffer is dispensed onto the membrane center and is absorbed completely.**
- **If the eluting DNA fragments >5kb, using the pre-heated EB Buffer (55~60℃) or incubating the tubes at 55~60℃ for 3 mins will increase the DNA yield.**
- **The recovery efficiency will decrease if the pH is not at the range of pH 7.0 to 8.5. And the product should be stored at -20℃ to avoid DNA degrading.**