FavorPrep™
GEL / PCR DNA Clean-Up Kit

User Manual

Cat. No.: FAGCK 100 (100 Preps)
FAGCK 300 (300 Preps)

For Research Use Only

Introduction

Gel/PCR Purification Kit is designed to recover or concentrate DNA fragments (50 bp - 10 kb) from agarose gel, PCR or other enzymatic reaction. The method use a chaotropic salt, guanidine thiocyanante to dissolve agarose gel and denature enzymes, and then DNA fragments in chaotropic salt are bonded to glass fiber matrix of the spin column(1). The contaminants are washed with a wash buffer containing ethanol, than the purified DNA fragments are eluted by low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides could be effectively removed from reaction mixture without phenol extraction and alcohol precipitation. Typically recoveries are 60-80% for gel extraction and 80-90% for PCR clean up.
The entire procedure can be completed in 20 minutes and the eluted DNA is ready to use in restriction digestion, ligation, PCR, and sequencing reaction.

Quality Control

The quality of Gel/PCR Purification Kit is tested on a lot-to-lot basis. The efficiency of DNA recovery are tested by isolation of DNA fragments of various sizes from either aqueous solution or agarose gel. The Purified DNA is checked by agarose gel analysis.

Sample: up to 300 mg of agarose gel
up to 100µl of reaction solution
Recovery: 60% ~ 80% for Gel extn
80% ~ 90% for PCR clean
Format: spin column
Operation time: 20 min
Elution volume: 20-40 µl

Kit Contents

Name	FAGCK 100 (100preps)	FAGCK 300 (300preps)
FAGX Buffer	80 ml	240 ml
Wash Buffer*	25 ml	50 ml
Elution Buffer	6 ml	30 ml
FAGX column	100 pcs	300 pcs
2 ml Collection tube	100 pcs	300 pcs

* add 100 / 200 ml ethanol(96-100%) to Wash Buffer prior to initial use.

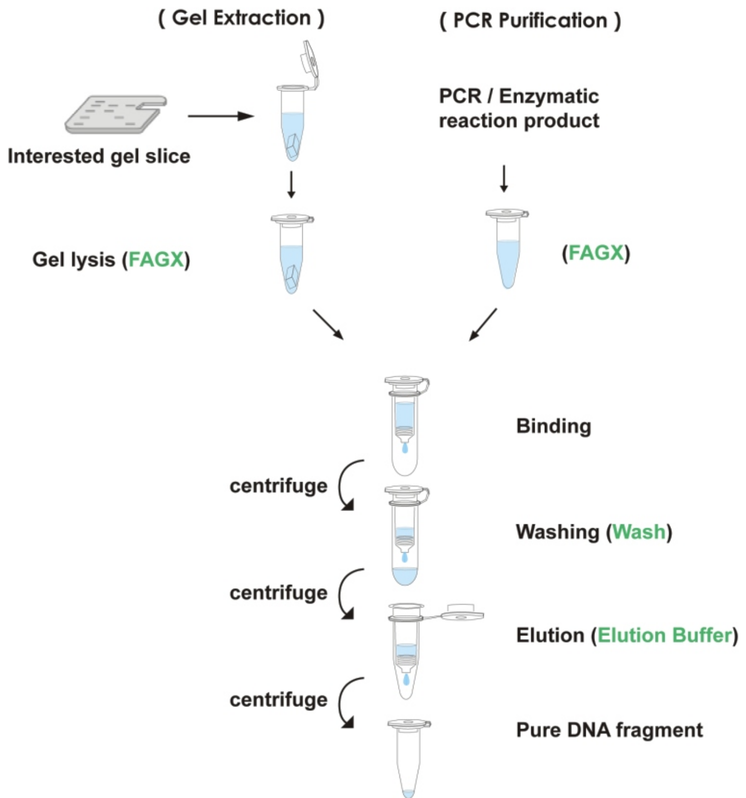
Caution

FAGX Buffer contain guanidine thiocyanante which is harmful and irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Brief procedure



Gel Extraction Protocol

Step 1

Gel Dissociation

- Excise the agarose gel slice containing relevant DNA fragments and remove extra agarose to minimize the size of the gel slice (It is better that use TAE buffer to make the gel than TBE buffer, because TBE buffer maybe will affect the downstream experiment).
- Transfer up to 300 mg of the gel slice into a microcentrifuge tube (not provided).
- Add **500µl of FAGX Buffer** to the sample and mix by vortexing.
- Incubate at 55-60°C for 10-15 minutes until the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 min.
- **Cool down the dissolved sample mixture to room temperature.**

Step 2

DNA Binding

- Place a **FAGX Column** in a **2 ml Collection Tube**.
- Apply 800µl of the sample mixture from previous step into the FAGX Column.
- Centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FAGX Column back in the Collection Tube.
- If the sample mixture is more than 800µl, repeat this **DNA Binding Step**.

Step 3

Wash

Add **600µl of Wash Buffer (ethanol added)** in the FAGX Column.

- Centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FAGX Column back in the Collection Tube.
- Centrifuge again for 3 minutes at full speed (13,000 rpm) to dry the column matrix.

Step 4

DNA Elution

Transfer dried column on a new microcentrifuge tube (not provided).

- Add **15-50μl of Elution Buffer** or water in the center of the column matrix.
- Stand for 3 minutes until Elution Buffer or water is absorbed by the matrix.
- Centrifuge for 3 minutes at full speed to elute purified DNA.

PCR Clean Up Protocol

Step 1

Sample preparation

- Transfer up to 100 μl reaction product to a microcentrifuge tube (not provided).
- Add **5 volumes of FAGX Buffer** to 1 volume of the sample and mix by vortexing.

Step 2

DNA Binding

- Place a **FAGX Column** in a **2 ml Collection Tube**.
- Apply the sample mixture from previous step into the FAGX Column.
- Centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FAGX Column back in the Collection Tube.

Step 3

Wash

- Add **600μl of Wash Buffer (ethanol added)** in the FAGX Column.
- Centrifuge at 13,000 rpm for 30 seconds.
- Discard the flow-through and place the FAGX Column back into the Collection Tube.
- Centrifuge again for 3 minutes at full speed (13,000 rpm) to dry the column matrix.

Step 4
DNA Elution

- Transfer dried column on a new microcentrifuge tube (not provided).
- Add **15-50μl of Elution Buffer** or water into the center of the column matrix.
- Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
- Centrifuge for 2 minutes at full speed to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Gel slice did not dissolve completely** • Gel slice was too big. If using more than 300 mg of gel slice, separate it into multiple tubes. • Raise temperature of incubation to 60℃ and extend incubation time. **Incorrect DNA Elution Step** • Ensure that Elution Buffer was added and absorbed to the center of FAGX Column matrix. **Incomplete DNA Elution** • If size of DNA fragments is larger than 8 kb, use preheated Elution Buffer (60-70℃) on Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** • After wash step, dry FAGX Column with additional centrifugation at top speed for 5 minutes or incubation at 60℃ for 5 minutes. **DNA was denatured (a smaller band appeared on gel analysis)** Incubate eluted DNA at 95℃ for 2 minutes, than cool down slowly to reanneal denatured DNA.