User Manual



FavorPrep™ 96-Well GEL Purification Kit

- For 96-well high-throughput purification of DNA from agarose gel

For Research Use Only

Kit contents:

Cat. No.: (Q'ty)	FAGKE 96001 (1 plate)	FAGKE 96002 (2 plates)	FAGKE 96004 (4 plates)
FAGP Buffer	70 ml	140 ml	140 ml × 2
Wash Buffer (conenttate)	* 17.5 ml	** 35 ml	** 35 ml × 2
Elution Buffer	30 ml	60 ml	60 ml × 2
Filter Plate (96-Well DNA Binding plate)	1 plate	2 plate	4 plate
Collection Plate (96-Well 2 ml Plate)	3 plates	6 plates	12 plates
Elution Plate (96-Well PCR plate)	1 plates	2 plates	4 plates
Adhesive Film	2 pcs	4 pcs	8 pcs

^{*, **} Add ethanol (96 \sim 100 %) to Wash Buffer when first use.

Storage:

All component of FavorPrep™ 96-Well GEL Purification Kit should be stored at room temperature (15 - 25 °C).

Preparation of working buffers

Add ethanol (96~100%) to Wash Buffer when first use.

Cat. No.	FAGKE96001	FAGKE96002 FAGKE96004
Ethanol volume for Wash Buffer	* 70 ml	** 140 ml

Quality control

The quality of FavorPrep™ 96-Well GEL Purification Kit is tested on a lot-to-lot basis. The purified DNA is checked by real-time PCR and capillary electrophoresis.

Specification:

Principle: Filter Plate (96-well plate, silica membrane)

Sample size: up to 200 mg agarose gel slice

DNA size: 65 bp ~ 10 kb

Processing: vacuum or centrifugation

Operation time: ≤ 45 minutes Typical recovery: 70% ~ 85%

DNA Binding capacity: up to 20 µg/ well Elution volume: 50 ~ 75 µl

Downstream application: Fluorescent or radioactive sequencing,

Restriction digestion, Library screening, Ligation, Labeling, Transformation.

Product Description:

FavorPrep™ 96-well GEL Purification Kit is designed for 96 wells high-throughput purification of DNA fragments or PCR products from agarose. The DNA are bound to the silica membrane of the DNA binding plate using a chaotropic salt buffer technique, and the primers, primer dimers, salts, nucleotides and proteins are removed from the membrane of the plate using a wash buffer. Then the highly pure DNA are eluted from the membrane in a low-ionic-strength buffer and are captured in a elution plate. The purified DNA are suitable for use directly in the downstream applications such as fluorescence or radio sequencing, restriction digestion, library screening, ligation, labeling, and transformation.

Additional materials required

For All Protocol:

- Pipets and pipet tips, sterile
- 96~100 % ethanol (for preparation of Wash Buffer).

For vacuum processing:

- A centrifugator is required for the clarification of lysate and for the alternative of elution step, capable of 5,600 ~ 6,000 X g, with a swing -bucket rotor and the adaptor for 96-well plates.
- A vacuum manifold for 96-well plate and a vacuum source reached to -12 inches Hg are required.

For centrifuge processing:

• A centrifugator is required, capable of 5,600 ~ 6,000 X g, with a swing-bucket rotor and the adaptor for 96-well plates.

Important notes:

- 1. Add ethanol (96~100%) to Wash Buffer when first use.
- 2. Check FAGP Buffer before use, Warm the Buffer at 60 °C for 5 minutes if any precipitate formd.
- 3. Components of this kit should be stored at $15 \sim 25$ °C.

Safety Information:

- 1. FAGP Buffer provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
- 2. **CAUTION:** FAGP Buffer contain quanidinium salts which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the preparation waste.

Kit Component: FAGP Buffer

Hazard contents Guanidinium thiocvanate

CAS-No. 593-84-0 EC-No. 209-812-1



Hazard statement(s) H302 + H312 + H332

Harmful if swallowed, in contact with

H314 Causes severe skin burns and eye

H412

Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P304 + P340 + P310

Do not breathe dust/ fume/ gas/ mist/ P260 vapours/spray.

P280

Wear protective gloves/ protective clothing/ eye protection/ face protection.

P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing.

Rinse skin with water/shower. IF INHALED: Remove person to fresh air

and keep comfortable for breathing. Immediately call a POISON CENTER/

doctor.

P305 + P351 + P338

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue

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Brief procedure:

• STEP 1 Sample preparation







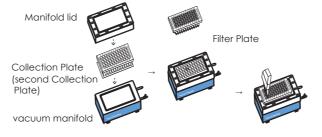
Incubate at 55 °C, 10 ~15 min, until gel slice dissolved completely

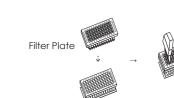


Mix well by pipetting

• STEP 2. Bind DNA to Filter Plate:

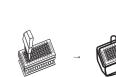
Vacuum processing	Centrifuge processing
 Transfer the sample mixture to Filter plate. Apply -12 inches Hg vacuum until the wells have emptied. 	 Transfer the sample mixture to Filter plate. Centrifuge at 4,500 ~ 6,000 x g for 2 min.





Collection Plate

(second Collection Plate)



• STEP 3. Wash the Filter Plate with Wash Buffer

• Add Wash Buffer. Apply vacuumat at -12 inches for 2 min



• Add Wash Buffer. Centrifuge at 5,600 - 6,000 x g for 10 min





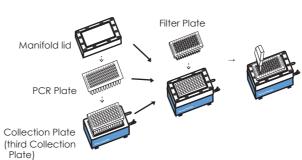
• STEP 4. Dry the membranes of the Filter Plate:

- Tap the Filter Plate tips on paper towel
- Return the Filter Plate and the second Collection Plate back to the manifold.
- Apply vacuum at -12 inches Hg for an additional 10 min.
- Stand the Filter plate on a clean paper towel at room

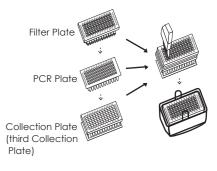
• STEP 5. DNA Elution:

- Add Elution Buffer to the Filter Plate. Stand for 3 min.
- Close the manifold volve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- Open the manifold valve to apply vacuum to elute DNA.

Alternative: If the consistent volume of elutes are recommend, use centrifuge processing for this elution step. (Page 3, STEP 6)



- Add Elution Buffer to the Filter Plate. Stand for 3 min.
- Centrifuge to elute DNA.



Protocol: vacuum processing

Please Read Important Notes and Safety Information before starting the following steps.

STEP 1. Sample preparation

- Transfer up to 200 mg of agarose gel (containing relevant DNA fragment) to each well of a 96-Well 2 ml Plate (provided, first 96-well, 2 ml plate).
- Add 500 µl of FAGP Buffer to each well, sealing with a adhesive film. Incubate at 55 °C for 10~15 min until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 min to make the sample mixture mix well with FAGP Buffer.

STEP 2. Bind DNA to Filter Plate

- Place a clean collection plate (provided, second Collection Plate) on the rack of vacuum manifold and cover the manifold lid. Place a Filter Plate (provided, 96-Well DNA binding plate) on top of the second Collection Plate.
- · Transfer the sample mixture to the Filter Plate and discard the first Collection Plate.
- · Apply vacuum at -12 inches Hg until the wells have emptied.
- · Release vacuum from the manifold.
- Discard the flow-through and return the Filter Plate and the second Collection Plate back to the manifold.

STEP 3. Wash the Filter Plate with Wash Buffer

- \cdot Add 500 μl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- · Apply vacuum at -12 inches Hg for 2 min.
- · Release vacuum from the manifold.
- · Discard the flow-through and return the Filter Plate and the second Collection Plate back to the manifold.

STEP 4. Dry the membranes of the Filter Plate

- Gently tap the tips of the Filter Plate on a clean paper towel to remove residual liquid.
- Return the Filter Plate to the second Collection Plate back to the manifold.
- · Apply vacuum at -12 inches Hg for an addition 10 min.
- · Release vacuum from the manifold.
- · Discard the second Collection Plate containing flow-through.

STEP 5. Elution - vacuum processing

Alternative: If the consistent volume of elutes are recommended, using centrifuge processing (STEP 6. Elution - centrifuge processing) for this elution step.

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, third collection plate) and fix plates on the rack of manifold. Cover the manifold lid and place the Filter Plate on the Elution Plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: the third Collection Plate)
- \cdot Add 50 \sim 75 μ I of Elution Buffer to the membrane center of the Filter Plate. Stand for 3 min.
- -- Note! The eluates averaged about 25 µl less than the adding volume of elution buffers. For example, adding 50 µl of Elution Buffer will recover ~ 25 µl of eluate.
- -- Note! Do not use Elution Buffer less than the suggested volume (< 50 μ l). It will lower the DNA yield.
- Note! For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.
- -- Note! Recovery of larger DNA fragments (> 5 kbp) can be increased by using pre-heated (70 °C) elution buffer.
- · Close the manifold volve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- · Open the manifold valve to apply vacuum to elute DNA.
- · Release vacuum from the manifold.
- Take out the Elution Plate (96-well PCR plate) and seal with an Adhesive Film (provided).
- · Store the DNA at -20 °C before use.

(Alternative) STEP 6. Elution - centerfuge processing

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, third collection plate) then place the Filter Plate on the Elution plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: Collection Plate)
- \cdot Add 50 \sim 75 μl of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
- -- Note! The eluates averaged about 25 μ l less than the adding volume of elution buffers. For example, adding 50 μ l of Elution Buffer will recover \sim 25 μ l of eluate
- -- Note! Do not use Elution Buffer less than the suggested volume (< 50 μl). It will lower the DNA yield.
- -- Note! For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.
- -- Note! Recovery of larger DNA fragments (> 5 kbp) can be increased by using pre-heated (70 °C) elution buffer.
- Place the combined plates in a rotor bucket and centrifuge at 5,600 6,000 x g for 5 min to elute DNA.
- Take out the Elution Plate (96-well PCR plate) and seal with an Adhesive Film (provided).
- · Store the DNA at -20 °C before use.

Protocol: centrifuge processing

Please Read Important Notes and Safety Information before starting the following steps.

STEP 1. Sample preparation

1A. For Gel DNA Extraction

- Transfer up to 200 mg of agarose gel (containing relevant DNA fragment) to each well of a 96-Well 2 ml Plate (provided, first 96-well, 2 ml plate).
- Add 500 µl of FAGP Buffer to each well, sealing with a adhesive film. Incubate at 55 °C for 10~15 min until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 min to make the sample mixture mix well with FAGP Buffer.

STEP 2. Bind DNA to Filter Plate

- · Place a Filter Plate (provided, 96-Well DNA binding plate) on a clean Collection Plate (provided, second collection plate).
- · Transfer the sample mixture to each well of the Filter Plate and discard the first Collection Plate.
- · Place the combined plates (Filter Plate + the second Collection Plate) in a rotor bucket and centrifuge at $5,600 \sim 6,000 \times g$ for 2 min
- · Discard the flow-through and return the Filter Plate back to the second Collection Plate.

STEP 3. Wash the Filter Plate with Wash Buffer

- · Add 500 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- Place the combined plates in a rotor bucket and centrifuge at $5,600 \sim 6,000 \times g$ for 10 min.
- Discard the flow-through and return the Filter Plate back to the Collection Plate.

STEP 4. Dry the membranes of the Filter Plate

Place the Filter Plate on top of a clean paper towel (not provided) and stand at room temperature for 5 min.

STEP 5. Elution

- Place a Elution Plate (provided, 96-Well PCR plate) on top of a clean Collection Plate (provided, third Collection Plate) then place the Filter Plate on the Elution plate. (top: Filter Plate, middle: 96-well PCR Plate, bottom: third Collection Plate)
- \cdot Add 50 \sim 75 μ I of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
- -- Note! The eluates averaged about 25 μ l less than the adding volume of elution buffers. For example, adding 50 μ l of Elution Buffer will recover \sim 25 μ l of eluate.
- -- Note! Do not use Elution Buffer less than the suggested volume (< 50 µl). It will lower the DNA yield.
- Note! For effective elution, make sure that Elution Buffer is dispensed on the membrane center and is absorbed completely.
- Note! Recovery of larger DNA fragments (> 5 kbp) can be increased by using pre-heated (70 °C) elution buffer.
- \cdot Place the combined plates in a rotor bucket and centrifuge at $5,600 \sim 6,000$ x g for 5 min to elute DNA.
- Take out the Elution Plate (96-well PCR plate) and seal with a Adhesive Film (provided).
- · Store the DNA at -20 °C before use.

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