User Manual



FavorPrep™ Gel Purification 96-Well Kit

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

For Research Use Only

Kit contents:

Cat. No.: (Qanity)	FAGP107A (1 plate)	FAGP107B (2 plates)	FAGP107C (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 (96~100 %) to Wash Buffer when first use.

Storage:

All component of FavorPrep™ Gel Purification 96-Well 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.	FAGP107A	FAGP107B FAGP107C
Ethanol volume for Wash Buffer	* 70 ml	** 140 ml

Quality control

The quality of FavorPrep™ Gel Purification 96-Well 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 mins 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 $^{\text{TM}}$ Gel Purification 96-Well 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).
- 96-Well PCR Rack

For vacuum processing:

- A centrifuge capable of reaching a minimum speed of 5,600~ 6,000 xg with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).
- A vacuun manifold for 96-well plate and a vaccum source reached to -12 inches Hg are required.

For centrifuge processing:

• A centrifuge capable of reaching a minimum speed of 5,600~ 6,000 xq with a microplate swinging-bucket rotor (capable of accommodating an 8.0 cm plate stack).

Important notes:

- 1. Add ethanol (96~100%) to Wash Buffer when first use.
- 2. Check Binding Buffer D1 before use, Warm the Buffer at 60°C for 5 mins if any precipitate formd.
- 3. Components of this kit should be stored at 15~25°C.
- 4. Ensure plates are tightly sealed with Adhesive Film to prevent sample loss or cross-well contamination. Never reuse adhesive

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

Danger

Hazard statement(s) H302 + H312 + H332

Harmful if swallowed, in contact with

skin or if inhaled. H314 Causes severe skin burns and eye

H412 Harmful to aquatic life with long

lasting effects.

Precautionary statement(s)

P305 + P351 + P338

Do not breathe dust/ fume/ gas/ mist/ P260 vapours/spray. Wear protective gloves/ protective clothing/ eye protection/ face protection. P280

IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P301 + P312 + P330 P303 + P361 + P353 IF ON SKIN (or hair): Take off

immediately all contaminated clothing. Rinse skin with water/shower. P304 + P340 + P310

IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/

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

rinsing.

Brief procedure:

• STEP 1 Sample preparation







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



Mix well by pipetting

• STEP 2. Bind DNA to Filter Plate:

vacuum manifold

Vacuum processing Transfer the sample mixture to Filter plate. Apply -12 inches Hg vacuum until the wells have emptied.		Centrifuge processing Transfer the sample mixture to Filter plate. Centrifuge at 4,500~6,000 xg for 2 mins.	
	Collection Plate (second Collection		

• STEP 3. Wash the Filter Plate with Wash Buffer

• Add Wash Buffer. Apply vacuum at -12 inches for 2 mins.



• Add Wash Buffer. Centrifuge at 5,600~6,000 xg for 10 mins.



Collection Plate (third Collection Plate)

Collection Plate

(second Collection Plate)





• 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 mins.
- Stand the Filter plate on a clean paper towel at room

• STEP 5. DNA Elution:

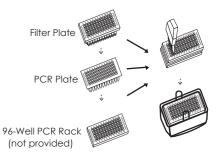
- Add Elution Buffer to the Filter Plate. Stand for 3 mins.
- Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.

Open the manifold valve to apply vacuum to elute DNA.

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



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



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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 mins until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 mins 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 Collection Plate (second).
- Place the Filter Plate and a clean Collection Plate (provided, third collection plate) to the manifold.

STEP 3. Wash the Filter Plate with Wash Buffer

- · Add 500 µl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- · Apply vacuum at -12 inches Hg for 2 mins.
- · Release vacuum from the manifold.
- · Discard the flow-through and return the 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 manifold.
- · Apply vacuum at -12 inches Hg for an addition 10 mins.
- \cdot Release vacuum from the manifold.
- · Discard the 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 an Elution Plate (provided, 96-Well PCR Plate) on the 96-Well PCR Rack (not provided) and fix plate onto manifold.
 Cover the manifold lid and place the Filter Plate on the Elution Plate (top: Filter Plate; middle: 96-Well PCR Plate; bottom: 96-Well PCR Rack).
- \cdot Add 50~75 μl of Elution Buffer to the membrane center of the Filter Plate. Stand for 3 mins.
- -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 ~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.
- · Close the manifold valve. 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 the combined Filter Plate and Elution Plate (provided, 96-Well PCR Plate) onto the 96-Well PCR Rack (not provided), forming a three-plate assembly in the following order: top – DNA Binding Plate; middle – Elution Plate; bottom – 96-Well PCR Rack.
- · Add 50~75 µl of Elution Buffer to the membrane center of the Filter Plate. Stand for 3 mins.
- -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 xa for 5 mins 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 mins until the gel slice dissolved completely. During the incubation, briefly shake the incubated plate for every 5 mins 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 5,600~6,000 xg for 2 min
- · Discard the Collection Plate (second).
- · Place the Filter Plate on a clean Collection Plate (provided, third 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 5,600~6,000 xg for 10 mins.
- · Discard the Collection Plate (third).

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 mins.

STEP 5. Elution

- Place the combined Filter Plate and Elution Plate (provided, 96-Well PCR Plate) onto the 96-Well PCR Rack (not provided), forming a three-plate assembly in the following order: top – DNA Binding Plate; middle – Elution Plate; bottom – 96-Well PCR Rack.
- \cdot Add 50~75 μ I of Elution Buffer to the membrane center of the Filter Plate. Stand for 3 mins.
- -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 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 assembled plate set in a rotor bucket and centrifuge at 5,600~6,000 xg for 5 mins 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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