User Manual



FavorPrep™ Gel/PCR Purification 96-Well Kit

For Research Use Only

 For 96-well high-throughput purification of DNA from agarose gel, PCR mixture or other enzymatic reaction mixture

Kit contents:

Cat. No.: (Qanity)	FAGC107A (1 plate)	FAGC107B (2 plates)	FAGC107C (4 plates)
Binding Buffer D1	70 ml	140 ml	140 ml × 2
Wash Buffer (concentrate)	17.5 ml	35 ml ▲	35 ml × 2▲
Elution Buffer	30 ml	60 ml	60 ml × 2
Filter Plates (96-Well DNA Binding plate	1 plate	2 plates	4 plates
Collection Plate (96-Well 2 ml Plate)	3 plates	6 plates	12 plates
Elution Plate (96-Well PCR plate)	1 plate	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/PCR 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.	FAGC107A	FAGC107B FAGC107C
Ethanol volume for Wash Buffer	■ 70 ml	▲ 140 ml

Quality control

The quality of FavorPrep™ Gel/PCR 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

up to 100 µl PCR or other enzymatic reaction mixture

DNA size: 65 bp~10 kb

Processing: vacuum or centrifugation

Operation time: ≤45 mins for gel DNA extraction

≤35 mins for PCR clean up

Typical recovery: 75%~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™ Gel/PCR Purification 96-Well Kit is designed for 96 wells high-throughput purification of DNA fragments or PCR products from agarose, PCR mixtures or enzymatic reaction mixtures. 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 radiosequencing, 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 xg 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.
- 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.
- Ensure plates are tightly sealed with Adhesive Film to prevent sample loss or cross-well contamination. Never reuse adhesive film

Safety Information:

Hazard contents

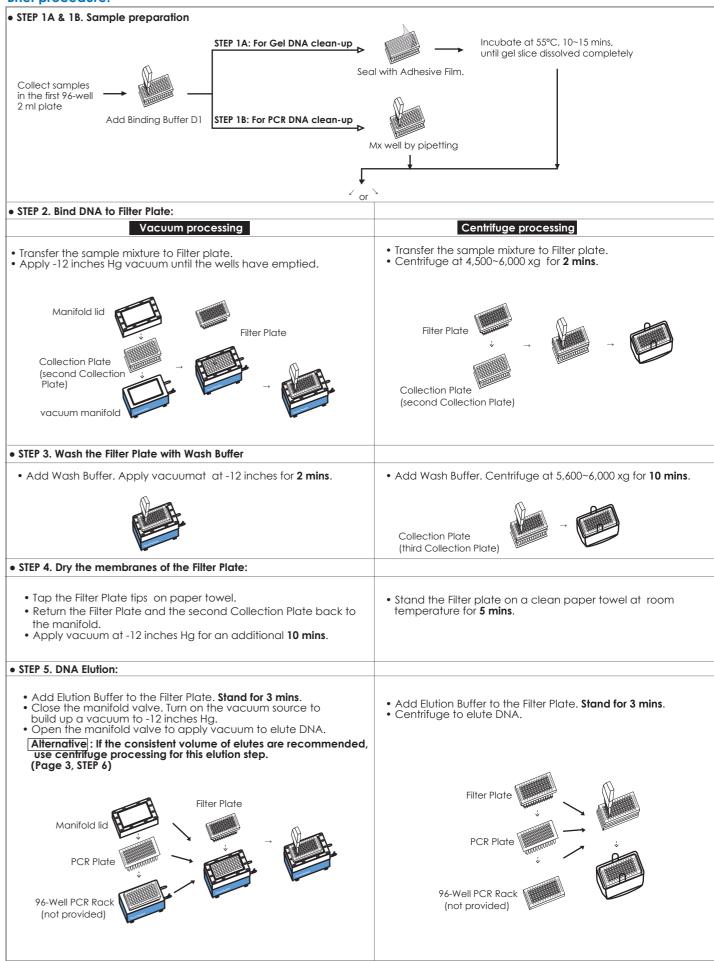
Kit Component: Binding Buffer D1

- 1. Binding Buffer D1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
- CAUTION: Binding Buffer D1 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.

Guanidinium thiocyanate CAS-No. 593-84-0 Dange EC-No. 209-812-1 Hazard statement(s) H302 + H312 + H332 Harmful if swallowed, in contact with skin or if inhaled. H314 Causes severe skin burns and eye damaae. Harmful to aquatic life with long H412 lasting effects. Precautionary statement(s) P260 Do not breathe dust/ fume/ gas/ mist/ 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 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. P303 + P361 + P353 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ P304 + P340 + P310 doctor IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue P305 + P351 + P338 rinsing.

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Brief procedure:



1

Protocol: vacuum 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 Binding Buffer D1 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 Binding Buffer D1.

1B. For PCR/enzymatic reaction DNA Clean-Up

- Transfer 10~100 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first Collection Plate).
- Add 5 volumes of Binding Buffer D1 Buffer to each well and mix completely by pipetting.
- -For example, add 500 $\stackrel{-}{\mu}l$ of Binding Buffer D1 to 100 μl of sample.

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

- \cdot Add 500 μl of Wash Buffer (ethanol added) to each well of the Filter Plate.
- · Apply vacuum at -12 inches Hg for 2 mins.
- \cdot 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 min.
- · 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 $50\sim75\,\mu$ l 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.
- Place the combined plates 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 an Adhesive Film (provided).
- \cdot 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 Binding Buffer D1 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 min to make the sample mixture mix well with Binding Buffer D1.

1B. For PCR/ enzymatic reaction DNA Clean-Up

- Transfer 10~100 µl of sample to each well of a Collection Plate (provided, 96-well 2 ml plate; first Collection Plate).
- Add 5 volumes of Binding Buffer D1 Buffer to each well and mix completely by pipetting.
- -For example, add 500 μl of Binding Buffer D1 to 100 μl of sample.

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 $5,600\sim6,000 \times g$ for 2 mins.
- 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

- \cdot 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 μl of Elution Buffer 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 ~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 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.