User Manual

FavorPrep™ 96-well Gel/ PCR Clean-Up Kit

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

For Research Use Only

Kit contents:

Cat. No.: (Q'ty)	FAPKE 96001 (1 plate)	FAPKE 96002 (2 plates)	FAPKE 96004 (4 plates)
Binding Buffer D1	70 ml	140 ml	140 ml x 2
Wash Buffer (concentrate)	17.5 ml ■	35 ml ▲	35 ml x 2 ▲
Elution Buffer	30 ml	60 ml	60 ml x 2
Filter Plate (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™ 96-Well Gel/ PCR Clean-Up 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.	FAPKE 96001	FAPKE 96002 FAPKE 96004
Ethanol volume for Wash Buffer	■ 70 ml	▲ 140 ml

Quality control

The quality of FavorPrep™ 96-Well Gel/ PCR Clean-Up 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 minutes for gel DNA extraction
≤35 minutes 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™ 96-well Gel/ PCR Clean-Up 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).

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 vacuun 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 Binding Buffer D1 before use, Warm the Buffer at 60°C for 5 minutes if any precipitate formd.
3. Components of this kit should be stored at 15~25°C.

Safety Information:

1. Binding Buffer D1 provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. ***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.***

Kit Component: **Binding Buffer D1**

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 damage.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P260	Do not breathe dust/ fume/ gas/ mist/ 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.
P304 + P340 + P310	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 rinsing.

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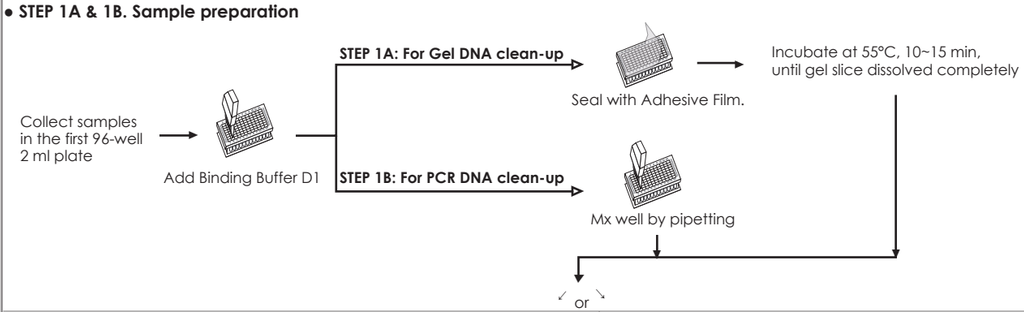
Brief procedure:

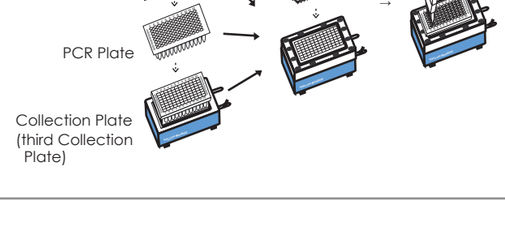
• STEP 1A & 1B. Sample preparation



• STEP 2. Bind DNA to Filter Plate:

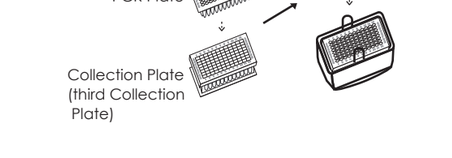
Vacuum processing

- Transfer the sample mixture to Filter plate.
- Apply -12 inches Hg vacuum until the wells have emptied.



Centrifuge processing

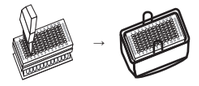
- Transfer the sample mixture to Filter plate.
- Centrifuge at 4,500~6,000 x g for **2 min**.



• 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 temperature for **5 min**.

• STEP 5. DNA Elution:

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

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 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 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 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

- 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)
- 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 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.
- 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 an 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)
- 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 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 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 Binding Buffer D1 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 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 5,600~6,000 x 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 5,600~6,000 x 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)
- 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 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 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 a Adhesive Film (provided).
- Store the DNA at -20°C before use.