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 (96~100 %) 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 15~25°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 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 damage. |
| H412 | Harmful to aquatic life with long lasting effects. |

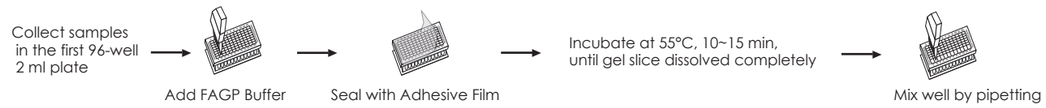
Precautionary statement(s)

| | |
|---|---|
| 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. |



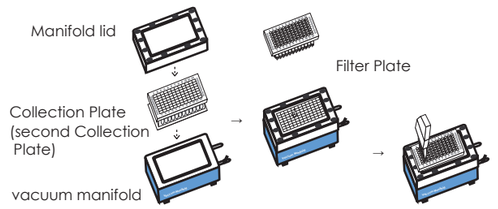
## Brief procedure:

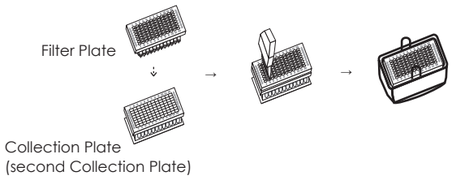
**• STEP 1 Sample preparation**




**• STEP 2. Bind DNA to Filter Plate:**

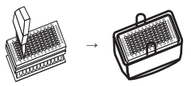
| Vacuum processing | Centrifuge processing |
|---|---|
| • Transfer the sample mixture to Filter plate.<br>• Apply -12 inches Hg vacuum until the wells have emptied. | • Transfer the sample mixture to Filter plate.<br>• Centrifuge at 4,500~6,000 x g for **2 min**. |




**• STEP 3. Wash the Filter Plate with Wash Buffer**

| | |
|---|---|
| • Add Wash Buffer. Apply vacuum at -12 inches for **2 min** | • Add Wash Buffer. Centrifuge at 5,600~6,000 x g for **10 min** |

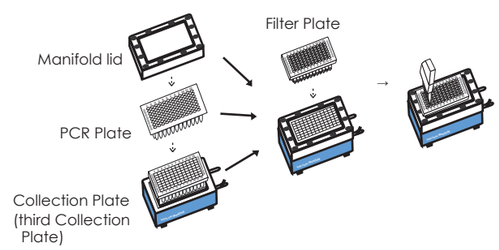


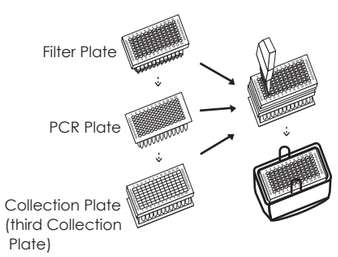
**• STEP 4. Dry the membranes of the Filter Plate:**

| | |
|---|---|
| • Tap the Filter Plate tips on paper towel<br>• Return the Filter Plate and the second Collection Plate back to the manifold.<br>• 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**.<br>• Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.<br>• Open the manifold valve to apply vacuum to elute DNA.<br>**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**.<br>• 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**

- 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 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)
- 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 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 5,600~6,000 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.