## **User Manual**



# FavorPrep™ 96-well PCR Clean-Up Kit

enzymatic reaction mixture

For Research Use Only

#### Kit contents:

Cat. No.: (Q'ty)	FACKE 96001 (1 plate)	FACKE 96002 (2 plates)	FACKE 96004 (4 plates)
FAPC Buffer	60 ml	120 ml	120 ml x 2
Wash Buffer (concentrate)	15 ml	30 ml ▲	30 ml x 2 ▲
Elution Buffer	15 ml	30 ml	30 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 plates

■, ▲: Add ethanol (96 ~ 100 %) to Wash Buffer when first use.

All component of FavorPrep™ 96-Well 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.	FACKE 96001	FACKE 96002 FACKE 96004
Ethanol volume for Wash Buffer	■ 60 ml	▲120 ml

#### **Quality control**

The quality of FavorPrep<sup>TM</sup> 96-Well 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: 10 ~ 100 µl of PCR mixture or other enzymatic reaction mixture.

DNA size: 65 bp ~ 10 kbp

Plate applicability: vacuum or centrifugation

Operation time: < within 45 min/96 preparations DNA Binding capacity: up to 20 µg/ well

Elution volume: 50 ~ 75 µl

A260/A280: 1.7~1.8

Downstream application: Fluorescent or radioactive sequencing, Restriction digestion, Library screening,

Ligation, Labeling, Transformation.

## **Product Description:**

FavorPrep™ 96-well PCR Clean-Up Kit is designed for 96 wells high-throughput purification of PCR products or DNA fragments from 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 an 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 a, 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  $5,600 \sim 6,000 \times g$ , with a swing-bucket rotor and the adaptor for 96-well plates.

### Important notes:

- 1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
- 2. Add ethanol (96~100%) to Wash Buffer when first use.
- 3. Check FAPC Buffer before use, Warm FAPC Buffer at 60 °C for 5 minutes if any precipitate formed.
- 4. Components of this kit should be stored at  $15 \sim 25$  °C.

#### **Safety Information:**

FAPC Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.

## Kit Component: FAPC Hazard contents

Guanidine hydrochloride

CAS-No. 50-01-1 EC-No. 200-002-3

Warning

lazard statement(s) H302 + H332

Harmful if swallowed or if inhaled.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Precautionary statement(s)

P261 Avoid breathing dust/ fume/ gas/

mist/vapours/spray.

P301 + P312 + P330 IF SWALLOWED: Call a POISON

CENTER/ doctor if you feel unwell.

Rinse mouth.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact

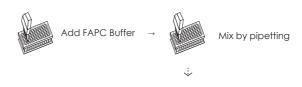
lenses, if present and easy to do.

Continue rinsing.

## **Brief procedure:**

### • STEP 1. Sample preparation

Transfer samples in a Collection Plate (firet Collection Plate)



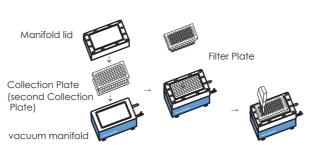
Vacuum protocol or Centrifuge protocol

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.





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, Centrifuae at 5,600 - 6,000 x g for **10 min** 



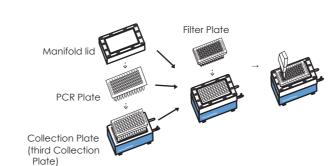
## • STEP 4. Dry the membranes of the Filter Plate:

- 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

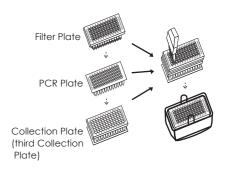
## • STEP 5. DNA Elution:

- 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



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## Protocol: vacuum processing

Please Read Important Notes and Additional Material Required before starting the following steps.

#### STEP 1. Sample preparation

- 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 FAPC Buffer to each well and mix completely by pipetting.
- -- For example, add 500 µl of FAPC Buffer 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.
- $\cdot$  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  $\mu l$  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.
- $\cdot$  Apply vacuum at -12 inches Hg for an addition 10 min.
- $\cdot$  Release vacuum from the manifold.
- $\cdot$  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  $\mu$ 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 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  $^{\circ}$ C) elution buffer.
- · Close the manifold valve. Turn on the vacuum source to build up a vacuum to -12 inches Hg.
- · Open the manifold volve 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  $\sim$  75  $\mu$ l of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
- -- Note! The eluates averaged about 25  $\mu$ l less than the adding volume of elution buffers. For example, adding 50  $\mu$ l of Elution Buffer will recover  $\sim$  25  $\mu$ l of eluate.
- -- Note! Do not use Elution Buffer less than the suggested volume (< 50 µl). It will lower the DNA vield.
- 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 Additional Material Required before starting the following steps.

### STEP 1. Sample preparation

- 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 FAPC Buffer to each well and mix completely by pipetting.
- -- For example, add 500 µl of FAPC Buffer 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.
- $\cdot$  Place the combined plates (Filter Plate + the second Collection Plate) in a rotor bucket and centrifuge at 5,600  $\sim$  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

- $\cdot$  Add 500  $\mu l$  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  $\mu l$  of RNase-free Water to the membrane center of the Filter Plate. Stand for 3 min.
- -- Note! The eluates averaged about 25  $\mu$ l less than the adding volume of elution buffers. For example, adding 50  $\mu$ l of Elution Buffer will recover  $\sim$  25  $\mu$ 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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