

# FAVORGEN

*For research use only*

## **FavorPrep™**

### **96-Well Plasmid Kit**

## **User Manual**

**Version 2 .2008.**

**Cat. No. FAPWE 001 (4 plates)**

**FAPWE 002 (10plates)**

*Store at room temperature (15 - 25 °C )*

For research used only

## **Introduction**

FavorPrep™ 96-well Plasmid DNA Extraction kit is designed for isolation of plasmid or cosmid DNA from 1- 5 ml of bacterial cultures. The procedure uses the modified alkaline lysis to get cleared lysate from bacteria cultures. With the suitable binding condition provided by this system, the plasmid DNA in the cleared lysate binds to glass fiber matrix in the 96-well Plasmid Binding Plate. The contaminants are washed with an ethanol-contained wash buffer and finally, the purified plasmid DNA is eluted by low salt Elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be done within 30 minutes and the purified plasmid DNA is ready for restriction digestion, ligation, PCR, and sequencing reaction.

## **Specification:**

**Sample Size:** 1~5 ml bacteria cultured

**Binding Capacity:** up to 20 µg/ well

**Operation:** centrifuge/ Vacuum manifold

**Handling Time:** within 30 minutes

**Application:** Fluorescent or radioactive sequencing  
Restriction digestion  
Library screening  
Ligation  
Transformation

## Kit Contents:

Name	FAPWE 001 (4 Plates)	FAPWE 002 (10Plates)
FAPD1 Buffer	100 ml	250 ml
FAPD2 Buffer	100 ml	250 ml
FAPD3 Buffer	140 ml	250 ml+100ml
Wash Buffer (concentrated)*	55 ml	50 ml x 3
Elution Buffer	40 ml	100 ml
RNase A (50 mg/ ml)	200 µl	500 µl
96-Well Plasmid plate	4 pcs	10 pcs
96-Well 0.35 ml Collection plate	4 pcs	10 pcs
Adhesive Film	8 pcs	20 pcs

\* Add 220 ml of ethanol to each Wash Buffer before first open.

## Important Notes:

1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
2. Briefly spin RNase A tube (provided) to remove drops from the inside of the lid and add RNase A to FAPD1 Buffer. Store FAPD1 Buffer at 4 °C after use.
3. Check FAPD2 Buffer before use to see if any precipitate formed, if yes, Warm FAPD2 Buffer in a 37 °C waterbath to dissolve precipitates.
4. To avoid acidification of FAPD2 Buffer from CO<sub>2</sub> in the air. Close the bottle immediately after use.
5. Add 220 ml of ethanol (96~100%) to each Wash Buffer before first open.

## Centrifuge Protocol

### Step 1

#### **Harvesting**

- Transfer **1-5 ml** of bacterial culture to each well of a 96-Well 2 ml Plate (not provided).
- Place in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5-10 minutes.
- Remove the medium completely by pipetting.

**Note:** Removal of residual medium is required for the lysis step.

### Step 2

#### **Resuspension**

- Add **250 µl** of **FAPD1 Buffer** (RNase A added) to each well of the plate and resuspend the cell pellet by vortexing (Seal with Adhesive Film) or pipetting.

**Note:** Complete cells resuspension is required for the lysis step.

### Step 3

#### **Lysis**

- Add **250 µl** of **FAPD2 Buffer**. Mix immediately by shaking (Seal with Adhesive Film) or pipetting.
- Stand for 2 minutes at room temperature until lysate clear.

### Step 4

#### **Neutralization**

- Add **350 µl** of **FAPD3 Buffer**. Seal with Adhesive Film. Mix immediately by shaking.
- Place in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5-10 minutes.

Step 5

**DNA Binding**

- Place a **96-Well Plasmid Plate** on top of a 96-Well 2 ml Plate (not provided).
- Transfer the clear lysate (supernatant) from **Step 4** to the **96-Well Plasmid Plate**. (about 350  $\mu$ l)
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5 min.
- Discard the flow-through and return the **96-Well Plasmid Plate** to the 96-Well 2 ml Plate.

Step 6

**Wash**

- Add **650  $\mu$ l of Wash Buffer (ethanol added)** into each well of the **96-Well Plasmid Plate**.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5 min. ·
- Discard the flow-through and return the **96-Well Plasmid Plate** back to the 96-Well 2 ml Plate.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for an additional 15 minutes to remove residual ethanol.

Step 7

**DNA Elution**

- Transfer the **96-Well Plasmid Plate** on a clean **96-Well 0.35ml collection plate (provided)**.
- Add **50~100  $\mu$ l of Elution Buffer** or ddH<sub>2</sub>O (pH8.0-8.5) into the membrane center of the **96-Well Plasmid Plate**.
- Stand the **96-Well Plasmid Plate** for 2 minutes until Elution Buffer or ddH<sub>2</sub>O has been absorbed by the membrane completely.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5 min to elute purified DNA into the 96-Well 0.35ml collection plate. Seal with Adhesive Film and store 4°C or -20°C.

## Vacuum Protocol

### Step 1

#### **Harvesting**

- Transfer **1-5 ml** of bacterial culture to each well of a 96-Well 2 ml Plate (not provided).
- Place in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5-10 minutes.
- Remove the medium completely by pipetting.

**Note:** Removal of residual medium is required for the lysis step.

### Step 2

#### **Resuspension**

- Add **250 µl** of **FAPD1 Buffer** (RNase A added) to each well of the plate and resuspend the cell pellet by vortexing (Seal with Adhesive Film) or pipetting.

**Note:** Complete cells resuspension is required for the lysis step.

### Step 3

#### **Lysis**

- Add **250 µl** of **FAPD2 Buffer**. Mix immediately by shaking (Seal with Adhesive Film) or pipetting.
- Stand for 2 minutes at room temperature until lysate clear.

### Step 4

#### **Neutralization**

- Add **350 µl** of **FAPD3 Buffer**. Seal with Adhesive Film. Mix immediately by shaking.
- Place in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5-10 minutes.

Step 5

**DNA Binding**

- Place a **96-Well Plasmid Plate** on top of the vacuum manifold.
- Transfer the clear lysate (supernatant) from **Step 4** into each well of the **96-Well Plasmid Plate**. (about 350  $\mu$ l)
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.

Step 6

**Wash**

- Add **650  $\mu$ l of Wash Buffer (ethanol added)** to each well of the **96-Well Plasmid Plate**.
- Apply vacuum at 10 inches Hg for 5 minutes until wells have emptied.
- Apply vacuum at 10 inches Hg for an additional 15 min and then incubate at 65°C for 15 min to remove residual ethanol.

Step 7

**DNA Elution**

- Transfer the **96-Well Plasmid Plate** on a clean **96-Well 0.35 ml collection plate (provided)**.
- Add **50~100  $\mu$ l of Elution Buffer** or ddH<sub>2</sub>O (pH8.0-8.5) into the membrane center of the **96-Well Plasmid Plate**.
- Stand the **96-Well Plasmid Plate** for 2 minutes until Elution Buffer or ddH<sub>2</sub>O has been absorbed by the membrane completely.
- Place the assembly plates in a rotor bucket and centrifuge at 4,500 – 6,000 xg for 5 min to elute purified DNA into the 96-Well 0.35ml collection plate. Seal with Adhesive Film and store 4°C or -20°C.