**Kit Contents:**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>FAPDE 004 (4 preps / sample)</th>
<th>FAPDE 100 (100 preps)</th>
<th>FAPDE 300 (300 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAPD1 Buffer</td>
<td>1.0 ml</td>
<td>25 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>FAPD2 Buffer</td>
<td>1.0 ml</td>
<td>25 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>FAPD3 Buffer</td>
<td>1.5 ml</td>
<td>35 ml</td>
<td>105 ml</td>
</tr>
<tr>
<td>W1 Buffer</td>
<td>2.0 ml</td>
<td>45 ml</td>
<td>135 ml</td>
</tr>
<tr>
<td>Wash Buffer [concentrate]</td>
<td>1.0 ml</td>
<td>20 ml</td>
<td>50 ml</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>0.5 ml</td>
<td>15 ml</td>
<td>35 ml</td>
</tr>
<tr>
<td>FAPD Column</td>
<td>4 pcs</td>
<td>100 pcs</td>
<td>300 pcs</td>
</tr>
<tr>
<td>Collection Tube</td>
<td>4 pcs</td>
<td>100 pcs</td>
<td>300 pcs</td>
</tr>
<tr>
<td>RNase A</td>
<td>0.1 mg</td>
<td>2.5 mg</td>
<td>7.5 mg</td>
</tr>
<tr>
<td>User Manual</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Preparation of Wash Buffer by adding ethanol (94 ~ 100%)

| Ethanol volume for Wash Buffer | 4 ml | 80 ml | 200 ml |

**Specification:**

- **Principle:** mini spin column (silica matrix)
- **Sample size:** 1 ~ 3 ml
- **Size of plasmid or construct:** < 15 kb
- **Operation time:** < 25 minutes
- **Typical Yield:** 20 ~ 30 µg
- **Binding capacity:** 60 µg/column
- **Column applicability:** centrifugation and vacuum

**Important Notes:**

1. Store RNase A at -20 °C upon receipt of kit.
2. Add 0.5 ml of FAPD1 Buffer to a RNase A tube, vortex the tube to mix well. Briefly spin the tube and transfer the total RNase A mixture back to the FAPD1 bottle, mix well by vortexing and store the FAPD1 buffer at 4 °C.
3. If precipitates have formed in FAPD2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of Wash Buffer by adding 96 ~100% ethanol (not provided) for first use.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

**General Protocol:**

*Please Read Important Notes Before Starting Following Steps.*

1. Transfer 1~3 ml of well-grown bacterial culture to a centrifuge tube (not provided).
2. Centrifuge the tube at 11,000 x g for 1 minute to pellet the cells and discard the supernatant completely.
3. Add 200 µl of FAPD1 Buffer (RNase A added) to the cell pellet and resuspend the cells completely by pipetting.
   - Make sure that RNase A has been added into FAPD1 Buffer when first use.
   - No cell pellet should be visible after resuspension of the cells.
4. Add 200 µl of FAPD2 Buffer and gently invert the tube 5 ~ 10 times. Incubate the sample mixture at room temperature for 2 ~ 5 minutes to lyse the cells.
   - Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate become clear.
   - Do not proceed the incubation over 5 minutes.
5. Add 300 µl of FAPD3 Buffer and invert the tube 5 ~ 10 times immediately to neutralize the lysate.
   - Invert immediately after adding FAPD3 Buffer will avoid asymmetric precipitation.
6. Centrifuge at full speed (~18,000 x g) for 5 min to clarify the lysate. During centrifugation, place a FAPD Column in a Collection Tube.
7. Transfer the supernatant carefully to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
   - Do not transfer any white pellet into the column.
8. Add 400 µl of W1 Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.

**Brief procedure:**

- **Well-grown bacterial culture**
  - Harvest bacterial cells
  - Lyse (FAPD2 Buffer)
  - Neutralize (FAPD3 Buffer)
- **Centrifuge, 11,000 x g, 30 sec**
  - Binding of plasmid
- **Centrifuge, ~18,000 x g, 3 min**
  - Washing (W1 Buffer)
- **Centrifuge, 11,000 x g, 30 sec**
  - Elution (Elution Buffer)
- **Centrifuge, ~18,000 x g, 1 min**
  - Pure plasmid
**Troubleshooting**

**Low yield**

Bacterial cells were not lysed completely
- Too many bacterial cells were used (OD600 > 10). Separate the bacterial culture into multiple tubes.
- After FAPD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.

Overgrown of bacterial cells
- Incubation time should not longer than 16 hours.

Bacterial cells were insufficient
- Ensure that bacterial cells have grown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.

Incorrect DNA elution step
- Ensure that Elution Buffer was added and absorbed to the center of the FAPD Column matrix.

Incomplete DNA Elution
- If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer (60~70°C) on elution step to improve the elution efficiency.

Incorrect preparation of Wash Buffer
- Ensure that the correct volume of ethanol (96 – 100 %) was added to and Wash Buffer prior to use.

**Eluted DNA does not perform well**

Residual ethanol contamination
- After Wash Step, dry the FAPD Column with an additional centrifugation at top speed (~18,000 x g) for 5 minutes or incubation at 60°C for 5 minutes.

Genomic DNA Contaminates

Lysate prepared improperly.
- Gently invert the tube after adding the FAPD2 Buffer. And the incubation time should not longer than 5 minutes.
- Do Not use overgrown bacterial culture.

**RNA Contaminates Plasmid DNA**

Insufficiency of RNase A activity in FAPD1 Buffer because of long-term storage
- Prior to using FAPD1 Buffer, ensure that RNase A was added. If RNase A added FAPD1 Buffer is out of date, add additional RNase A into FAPD1 Buffer to a concentration of 50 μg/ ml then store 4°C.
- Too many bacterial cells were used, reduce sample volume.

Smearing or degrading of Plasmid DNA

Nuclease contamination
- If used host cells have high nuclease activity (e.g., enA⁺ strains), perform the following optional Wash Step to remove residual nuclease.
  - a. After DNA Binding Step, add 400 μl of W1 Buffer into the FAPD Column and incubate for 2 minutes at room temperature.
  - b. Centrifuge at full speed (~18,000 x g) for 30 seconds.

**Plasmid DNA is not adequate for enzymatic digestions**

Eluted plasmid DNA contains residual ethanol
- Make sure you have discarded the flow-through after washing with Wash Buffer (Step 9) and centrifuged for an addition 3 minutes (Step 10).

**Denatured Plasmid DNA migrate faster than supercoiled form during electrophoresis**

Incubation in FAPD2 Buffer too long
- Do not incubate the sample longer than 5 minute in FAPD2 Buffer.

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9. Add 700 µl of Wash Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
- Make sure that ethanol (96-100%) has been added into Wash Buffer when first use.

10. Centrifuge at full speed (~18,000 x g) for an additional 3 minutes to dry the FAPD Column.
- Important step! The residual liquid should be removed thoroughly on this step.

11. Place the FAPD Column to a new 1.5 ml microcentrifuge tube (not provided).

12. Add 50 µl ~ 100 µl of Elution Buffer or ddH2O to the membrane center of the FAPD Column. Stand the column for 1 minute.
- Important step! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.

- **Note!** Do not Elute the DNA using less than suggested volume (50ul). It will lower the final yield.

13. Centrifuge at full speed (~18,000 x g) for 1 minute to elute plasmid DNA and store the DNA at -20°C.