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User Manual: # Plasmid Extraction Mini Kit

Cat. No.: DE 034s (4 preps)
DE 034 (100 preps)
DE 035 (300 preps)

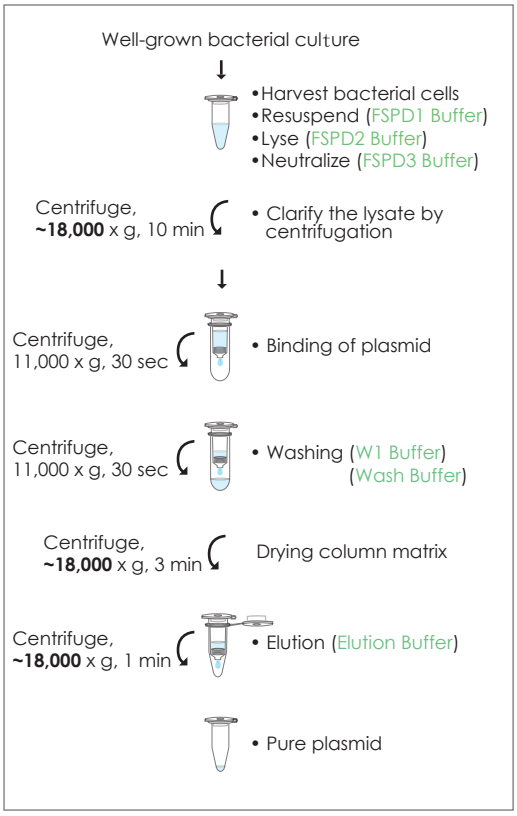
The Plasmid Extraction Mini Kit provides a rapid, phenol-free method for the extraction of high-purity plasmid DNA from bacterial culture such as E. coli. Silica membrane based DNA column is utilized in the purification process, and the extraction is carried out in three simple steps: binding/washing/elution. The plasmid DNA bound to the silica membrane, and the contaminants can be removed by wash buffer. The extracted DNA can be used in a variety of applications such as PCR, cloning, sequencing, in vitro transcription, Labeling, Restriction analysis and Transfection of cells.

(For Research Use Only)

Kit Contents:

Cat. No:	DE 034s-Mini (4 preps_sample)	DE 034 (100 preps)	DE 035 (300 preps)
FSPD1 Buffer	1.5 ml	30 ml	90 ml
FSPD2 Buffer	1.5 ml	30 ml	90 ml
FSPD3 Buffer	1.5 ml	40 ml	120 ml
W1 Buffer (concentrate) [a]	1.3 ml	35 ml	98 ml
Wash Buffer (concentrate) [b]	1.0 ml	20 ml	50 ml
Elution Buffer	0.5 ml	15 ml	35 ml
FSPD Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
RNase A	0,15 mg	3 mg	9 mg
User Manual	1	1	1
Preparation of W1 Buffer and Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for W1 Buffer [a]	0.5 ml	13 ml	36 ml
Ethanol volume for Wash Buffer [b]	4 ml	80 ml	200 ml

Brief procedure:



Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1 ~ 5 ml
Size of plasmid or construct:	< 15 kb
Operation time:	< 25 minutes
Typical Yield:	25 ~ 40 µg
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of FSPD1 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 FSPD1 bottle, mix well by vortexing and store the FSPD1 buffer at 4 °C.
3. If precipitates have formed in FSPD2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of W1 Buffer and 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~ 5 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 250 µl of FSPD1 Buffer (RNase A added) to the cell pellet and resuspend the cells completely by pipetting.**
   - Make sure that RNase A has been added into FSPD1 Buffer when first use.
   - No cell pellet should be visible after resuspension of the cells.
4. **Add 250 µl of FSPD2 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 350 µl of FSPD3 Buffer and invert the tube 5 ~ 10 times immediately to neutralize the lysate.**
   - Invert immediately after adding FSPD3 Buffer will avoid asymmetric precipitation.
6. **Centrifuge at full speed (~18,000 x g) for 10 min to clarify the lysate. During centrifugation, place a FSPD Column in a Collection Tube.**
7. **Transfer the suspernatant carefully to the FSPD 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 FSPD 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 W1 Buffer when first use.

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9. Add 700 µl of Wash Buffer to the FSPD 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 FSPD Column.

• **Important step !** The residual liquid should be removed thoroughly on this step.

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

12. Add 50 µl ~ 100 µl of Elution Buffer or ddH_2O to the membrane center of the FSPD 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.

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 FSPD3 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 FSPD Column matrix.

Incomplete DNA Elution

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

Incorrect preparation of W1 Buffer and Wash Buffer

- Ensure that the correct volume of ethanol (96 ~ 100 %) was added to W1 Buffer and Wash Buffer pior to use.

Eluted DNA does not perform well

Residual ethanol contamination

- After Wash Step, dry the FSPD 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 FSPD2 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 FSPD1 Buffer because of long-term storage

- Prior to using FSPD1 Buffer, ensure that RNase A was added. If RNase A added FSPD1 Buffer is out of date, add additional RNase A into FSPD1 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 residuary nuclease.
  a. After DNA Binding Step, add 400 µl of W1 Buffer into the FSPD Column and incubate for 2 minutes at room temperature.
  b. Centrifuge at full speed (~18,000 xg) for 30 seconds.
  c. Proceed to step 9.

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 supercoilded form during electrophoresis

Incubation in FSPD2 Buffer too long

- Do not incubate the sample longer than 5 minute in FSPD2 Buffer

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Protocol (for isolation of plasmid from gram-positive bacteria)

1. Transfer 1-5 ml of well-grown bacteria culture to a microcentrifuge tube (not provided).

2. Descend the bacteria by centrifuging for 1-2 min and discard the supernatant completely.

3. **Add 250 µl of** FSPD1 Buffer containing RNase A and lysozyme (Provided by user) (for final concentration of 1 mg/ml) **to the pellet and resuspend the cells completely by pipetting.** And incubate the sample mixture at 37 ° C for 15 min.

   - Make sure that RNase A has been added into FSPD1 Buffer when first open.

Make sure that lysozyme has been added into FSPD1 buffer for final concentration of 1mg/mL

No cell pellet should be visible after resuspension of the cells.

4. **Add 250 µl of FSPD2 Buffer and gently invert the tube 5 times to lyse the cells and incubate at room temperature for 2 min.**

Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate beccome clear.
Do not proceed this step over 5 min.

5. **Add 350 µl of FSPD3 Buffer and invert the tube 5 times immediately but gently.** Invert immediately after addind FSPD3 Buffer will avoid asymmetric precipitation.

6. **Centrifuge for 10 min. During centrifuging, place a FSPD Column in a Collection Tube.**

7. **Transfer the suspernatant carefully to FSPD Column. Centrifuge for 1 min then discard the flow-through.**

Do not transfer any white pellet into the column

8. **Add 400 µl of W1 Buffer to FSPD Column. Centrifuge for 1 min then discard the flow-through.** Make sure that ethanol (96-100 %) has been added into W1 Buffer when first open.

9. **Add 750 µl of Wash Buffer to FSPD Column. Centrifuge for 1 min then discard the flowthrough.** Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.

10. **Centrifuge for an additional 5 min to dry the column.**

Important step ! This step will remove the residual liquid completely that will inhibit subsequent enzymatic reaction.

11. **Place FSPD Column to a new 1.5 ml microcentrifuge tube (not provided).**

12. **Add 50 µl~100 µl of Elution Buffer preheated at 65°or ddH2O to the membrane center of FSPD Column. Stand the column for 1 min.**

Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.

13. **Centrifuge for 1 min to elute plasmid DNA.IMPORTANT: Do not elute the DNA using less than suggested volume (50 ul) It will lower the final yield.**
14. **Store plasmid DNA at 4 °C or -20 °C.**

Special Protocol-(for isolation of plasmid from Yeast)

1. Transfer 0.5~1.5 ml of well-grown yeast culture to a microcentrifuge tube (not provided).
2. Descend the cells by centrifuging for 1-2 min and discard the supernatant completely.
3. Add 250 µl of **FSPD1 Buffer** containing **RNase A** and **Zymolase** (provide by user) (for final concentration of 2 mg/ml) to the pellet and resuspend the cells completely by pipetting. And incubate the sample mixture at 37 ° C for 15 ~ 60 min.
   - Make sure that RNase A provided by this kit has been added into FSPD1 Buffer when first open.
   - Make sure that zymolase has been added into FSPD1 **for final concentration of 2 mg/ml**

   No cell pellet must be visible after resuspension of cells.
4. Add 250 µl of FSPD2 Buffer and gently invert the tube 5 times to lyse the cells and incubate at room temperature for 2 min.
5. Add 350 µl of FSPD3 Buffer and invert the tube 5 times immediately but gently. Invert immediately after addind FSPD3 Buffer will avoid asymmetric precipitation.
6. Centrifuge for 10 min. During centrifuging, place a FSPD Column in a Collection Tube.
7. Transfer the suspernatant carefully to FSPD Column. Centrifuge for 1 min then discard the flow-through.
   - Do not transfer any white pellet into the column.
8. Add 400 µl of W1 Buffer to FSPD Column. Centrifuge for 1 min then discard the flow-through. **Make sure**
   - **that ethanol (96-100 %) has been added into W1 Buffer when first open.**

9. **Add 750 µl of Wash Buffer to FSPD Column. Centrifuge for 1 min then discard the flowthrough.** Make sure that ethanol (96-100 %) has
   - been added into Wash Buffer when first open.

10. **Centrifuge for an additional 3 min to dry the column.**
    - Important step ! This step will remove the residual liquid completely that will inhibit subsequent enzymatic reaction.

11. **Place FSPD Column to a new 1.5 ml microcentrifuge tube (not provided).**
12. **Add 50 µl~100 µl of Elution Buffer preheated at 65°C or ddH2O to the membrane center of FSPD Column. Stand the column for 1 min.**
    - Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.
    - Important : Do not Elute the DNA using less than suggested volume (50ul). It will lower the final yield.
13. **Centrifuge for 1 min to elute plasmid DNA.**
14. **Store plasmid DNA at 4 ˚C or -20 ˚C.**