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PLASMID DNA EXTRACTION MINI KIT DE-034 – DE-035

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.

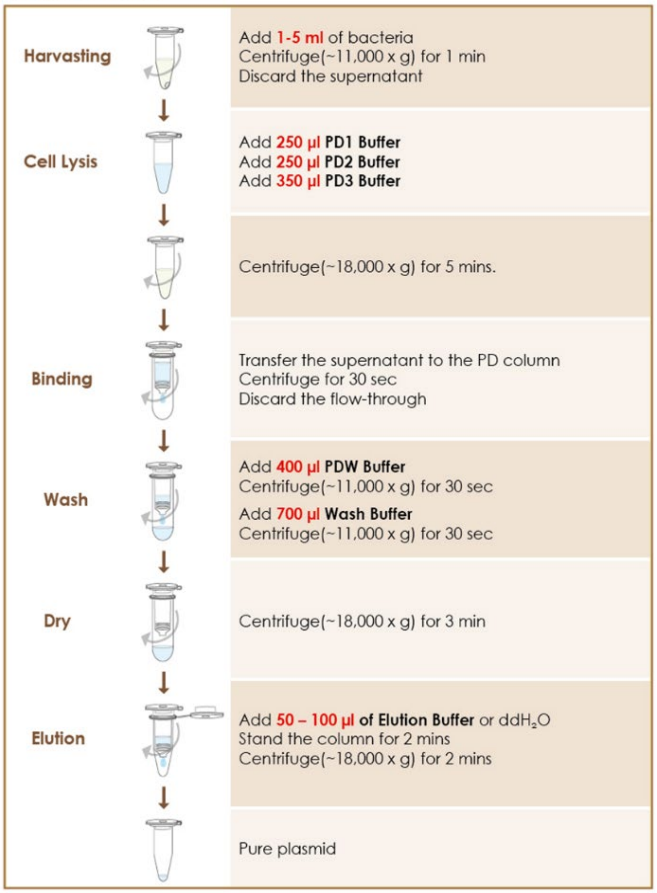
Kit Contents:

Cat. No:	DE 034s-Mini (4 preps_sample)	DE 034 (100 preps)	DE 035 (300 preps)
PD1 Buffer	1.5 ml	30 ml	90 ml
PD2 Buffer	1.5 ml	30 ml	90 ml
PD3 Buffer	1.5 ml	40 ml	120 ml
PDW 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
PD 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 to add to PDW buffer	0.5 ml	13 ml	36 ml
Ethanol Volume to add to Wash Buffer conc.	4 ml	80 ml	200 ml

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 vacuum

Brief procedure:



Important Notes:

1. Store RNase A at -20 °C upon receipt of the kit.
2. Add 0.5 ml of PD1 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 PD1 bottle, mix well by vortexing and store the PD1 buffer at 4 °C.
3. If precipitates have formed in PD2 Buffer, warm the buffer in 37°C water bath to dissolve precipitates.
4. Preparation of PDW 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.

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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).
   Centrifuge the tube at 11,000 x g for 1 minute to pellet the cells and discard the supernatant completely.
2. **Add 250 µl of PD1 Buffer (RNase A added)** to the cell pellet and resuspend the cells completely by pipetting.
   Note:
   - Make sure that RNase A has been added into PD1 Buffer when first use.
   - No cell pellet should be visible after resuspension of the cells.
3. **Add 250 µl of PD2 Buffer** and gently invert the tube 5 ~ 10 times. Incubate the sample mixture at room temperature for 2 ~ 5 minutes to lyse the cells.
   Note:
   - 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.

4. **Add 350 µl of PD3 Buffer** and invert the tube 5 ~ 10 times immediately to neutralize the lysate.
   Do not Vortex, vortex may shear genomic DNA
   - Invert immediately after adding PD3 Buffer will avoid asymmetric precipitation.

   centrifuge at full speed (~18,000 x g) for 10 min to clarify the lysate. During centrifugation, place a PD Column in a Collection Tube.

5. Transfer the supernatant carefully to the PD Column and centrifuge at 11,000 x g for 30 seconds.
   Discard the flow-through
   Place the PD column back into collection tube
   - Do not transfer any white pellet into the column.

6. **Add 400 µl of PDW Buffer** to the **PD** Column
   centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
   Place the PD column back into the Collection tube

7. Add **700 µl** of Wash Buffer to the PD 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.

8. Centrifuge at full speed (~ 18,000 x g) for an additional 3 minutes to dry the PD Column**.**
   - **Important step !**
     The residual liquid should be removed thoroughly on this step to avoid the inhibition of the enzymatic reaction.

9. Place the PD Column to a new **1.5 ml** microcentrifuge tube (not provided).

10. **Add 50 µl ~ 100 µl of** Elution Buffer or ddH2O to the membrane center of the PD Column.
    Stand the column for 2 minutes.

    NOTE:
    - 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.

11. **Centrifuge at full speed (~ 18,000 x g) for 1 minute to elute plasmid DNA and store the DNA at 4° for short Term or at -20 °C for longer term.**

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Special Protocol

(for isolation of plasmid from Yeast)

1. **Transfer 1 ~ 5 ml of well-grown yeast culture to a microcentrifuge tube (not provided).**
2. **Descend the cells by centrifuging at 5,000 x g for 5 min and discard the supernatant completely.**
3. **Add 250 µl of PD1 Buffer containing RNase A and lyticase (Provide by user) (for final concentration of 5 mg/ml) to the pellet and resuspend the cells completely by pipetting. And incubate the sample mixture at 37 ° C for 30 ~ 60 min.**

- Make sure that RNase A provided by this kit has been added into PD1 Buffer when first open.
- Make sure that lyticase has been added into PD1 for final concentration of 2 mg/ml
- No cell pellet should be visible after resuspension of the cells.

4. **Add 250 µl of PD2 Buffer and gently invert the tube 5 times to lyse the cells and incubate at room temperature for 10 min.**
5. **Add 350 µl of PD3 Buffer and invert the tube 5 times immediately but gently.**
   - Invert immediately after adding PD3 Buffer will avoid asymmetric precipitation.
6. **Centrifuge at 18,000 x g for 10 min. During centrifuging, place a PD Column in a Collection Tube.**
7. **Transfer the suspernatant carefully to the PD Column. Centrifuge at 18,000 x g for 1 min then discard the flow-through**
   - Do not transfer any white pellet into the column.
8. **Add 400 µl of PDW Buffer to the PD Column. Centrifuge for 1 min then discard the flow-through.**
9. **Add 700 µl of Wash Buffer to the PD Column. Centrifuge for 1 min then discard the flow-through.**
   - 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 the PD Column to a new 1.5 ml microcentrifuge tube (not provided).**
12. **Add 50 µl ~ 100 µl of 65 °C of Elution Buffer or ddH2O to the membrane center of the PD 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 the plasmid DNA.**
14. **Store plasmid DNA at 4 °C or -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 PD3 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 PD 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 PD 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 PD2 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 PD1 Buffer because of long-term storage

- Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A into PD1 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 PD 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

a. 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 PD2 Buffer too long

- Do not incubate the sample longer than 5 minute in PD2 Buffer

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