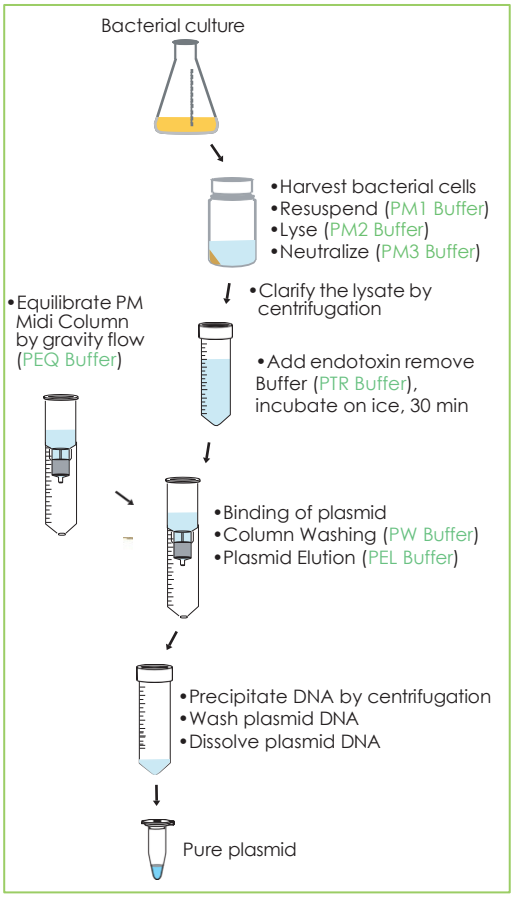
PLASMID DNA EXTRACTION MIDI PREP KIT (Endotoxin Free) DE-051EF

The Plasmid DNA Extraction Midi Kit Endotoxin free, is designed for rapid and efficient extraction of high quality plasmid DNA. With provided filter cartridges the bacteria lysates will be removed without centrifugation. Following a gravity-flow procedure, the plasmid DNA is bound to the resin, and the contaminants can be removed with wash buffer. After using this convenient kit, the purified plasmid DNA is suitable for downstream application such as transfection, in vitro transcription and translation, and all enzymatic modification.

Specifications:

Technology: Anion-exchange chromatography (gravity-flow column)
Lysate clarification: centrifugation
Sample Size: up to 60 ml of bacteria for high-copy number plasmid
up to 120 ml of bacteria for low-copy number plasmid
Plasmid or constructs range: 3kbp ~ 150kbp
Binding Capacity: 650 µg / Midi Column



Important Notes:

1. Store RNase A at -20 °C upon receipit of kit.
2. Add indicated volume or RNase A Solution ino PM1 buffer, mix well and store the PMI buffer at 4°C.
Transfer the total RNase A mixture back to the PM1 bottle, mix well by vortexing and store the PM1 buffer at 4 °C.
3. If precipitates have formed in PM2 Buffer, warm the buffer in 37°C waterbath to dissolve preciptates.
4. Prechill PM3 Buffer at 4 °C before starting.

Additional Requirements:

1. 50 ml tube
2. Refrigerated centrifuge capable of ≥ 5,000 x g and the centrifuge tube suitable for the centrifuge rotor
3. Isopropanol
4. 70% ethanol
5. TE buffer or ddH2O

Components	DE-051EF-s 2 preps sample	DE-051EF 25 preps
PEQ Buffer	12 ml	135 ml
PM1 Buffer*	20 ml	215 ml
PM2 Buffer	20 ml	215 ml
PM3 Buffer	20 ml	215 ml
PTR Buffer	6 ml	65 ml
PW Buffer	30 ml	270 ml + 60 ml
PEL Buffer	20 ml	215 ml
RNase A (solution)	100 ul	900 ul
PM Midi Column	2 pcs	25 pcs

Preparation of PM1 Buffer for the first use:

Cat.n.	DE-051EF-s 2 preps sample	DE-051EF 25 preps
Volume of RNase A Solution for PM1 Buffer	80 ul	860 ul

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36 Terry Drive
Trevose, PA 19048 - USA

General Protocol:

Please Read Important Notes Before Starting Following Steps.

Harvest bacterial cells

1. Harvest the cells by centrifugation at 4,500 ~ 6,000 x g at 4 ˚C for 10 min and discard the supernatant.

Equilibrate PM Midi Column

2. Place a PM Midi Column onto a 50 ml tube.
3. Equilibtate the PM Midi column by applying 5 ml of PEQ Buffer. Allow the column to empty by gravity flow and discard the filtrate.

Cell lysis and lysate neutralization

4. Add 8 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.
5. Add 8 ml of PM2 Buffer and mix gently by inverting the tube 5 times. -- Do not vortex to avoid shearing genomic DNA.
6. Incubate the sample mixture for 5 minutes at room temperature until lysate clears.
7. Add 8 ml of chilled PM3 Buffer and mix immediately by inverting the tube 10 ~15 times to neutralize the lysate. (Do not vortex !)

Note: •Make sure the density of cultured cell is optimal, the buffers volume (PM1, PM2, PM3) should be increased proportionally to the culture volume.
(ex. culture volume, 60 ~ 120 ml: PM1, 8 ml; PM2, 8 ml; PM3, 8 ml
culture volume, 120~ 240 ml: PM1, 16 ml; PM2, 16 ml; PM3, 16 ml)
Make sure cell pellet be suspended completely within Buffer PM1.
Mix the sample mixture completely after adding Buffer PM2 and Buffer PM3

Lysate clarification and endotoxin removal

8. **Centrifuge the tube at ≥ 5,000 x g at 4 °C 4 ˚C for 20 min. preferably centrifuge the tube at 15,000 ~ 20,000 x g at 4 ˚C for 15 minutes .**

-- If the supernatant still contains suspended matter, transfer the supernatant to a clean centrifuge tube and repeat this centrifugation step.

9. **Transfer the supernatant to a clean 50 ml Tube**

10. **Add 2,5 ml of PTR Buffer and mix gently by pipetting. Incubate the sample mixture on ice for 30 minutes After the incubation the sample mixture will become clear.**

Binding of plasmid

11. Transfer the sample mixture to the equilibrated **PM Midi column.** Allow the sample mixture to flow through the PM Midi Column by gravity flow and discard the filtrate.

Wash PM Midi Column

12. **Wash the PM Midi** column by applying 12.5 ml of PW Buffer. Allow PW Buffer to flow through the PM Midi Column by gravity flow and discard the filtrate.

Elution

13. **Place the PM Midi column** onto a clean 50 ml centrifuge tube (not provided). Add 8 ml of PEL Buffer to the PM Midi Column to elute the plasmid by gravity flow.

Precipitate plasmid DNA

14. **Transfer the eluate from step 13 to a centrifuge tube.** Add 0.75X volume of room temperature isopropanol to the eluate and mix well by inverting the tube 10 times. (ex: add 6 ml isopropanol to 8 ml eluate) Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.
15. **Centrifuge the tube at ≥ 5,000 x g at 4 ˚C for 30 min**. preferably centrifuge the tube at 15,000 ~ 20,000 x g at 4 ˚C for 20 minutes.

Wash and dissolve plasmid DNA

16. **Carefully remove the supernatant** and wash the plasmid pellet with 5 ml of room temperature 70% ethanol.
17. **Centrifuge the tube at ≥ 5,000 x g at 4 ˚C for 10 min**.
18. **Carefully remove the supernatant and invert the tube on paper towel for 3 minutes to remove residual ethanol**. Air-dry the plasmid pellet until the tube is completely dry. (Or incubate the plasmid pellet at 70 ˚C for 10 min.)
19. **Dissolve the plasmid pellet in a suitable volume (≥ 300 µl) of TE or ddH2O**. Note!

•Do not lose the DNA pellet when discard the supernatant.

-Make sure the DNA pellet adhesive lightly on the centrifuge tube.
-If the DNA pellet loose from tube, repeat the precipitation step again.

-Make sure the DNA is dissolved completely before measure the concentration.

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Troubleshooting

Low yield

Bacterial cells were not lysed completely

- Too many bacterial cells were used.
- After PM3 Buffer addition, break up the precipitate by inverting.
- DNA failed to precipitate or DNA pellet was lost after precipitation.
- DNA pellet was insufficiiently redissolved.

Purified DNA dose not perform well in downstream application

RNA contamination

- Make sure that that RNase A was has been added in PM1 Buffer when first using. If RNase A added PM1 Buffer is overdue, add additional RNase A.
- Too many bacterial cells were used, reduce the sample volume.

Genomic DNA contamination

- Do not use overgrown bacterial culture.
- During PM2 and PM3 Buffer addition, mix gently to prevent genomic DNA shearing.
- Lysis time was too long (over 5 minutes).

Too much salt residual in DNA pellet

Wash the DNA pellet twice with 70% ethanol.

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