FISHER MOLECULAR BIOLOGY

Plasmid Extraction Maxi Filter Endotoxin free Kit DE-055EFs & DE-055EF

The Plasmid DNA Extraction Maxi Filter endo ree Kit 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 remove 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.

Specification:

Technology: Anion-exchange chromatography (gravity-flow column)

Lysate clarification: Filtration

Sample Size: 120 - 240 ml of bacteria for high-copy number or low copy number plasmid

Plasmid or constructs range: 3kbp ~ 150kbp
Binding Capacity: 1.5 mg / Maxi Column
Yield: Up to 1500 µg / column
Handling time: less than 1.5 hours

Kit Contents:

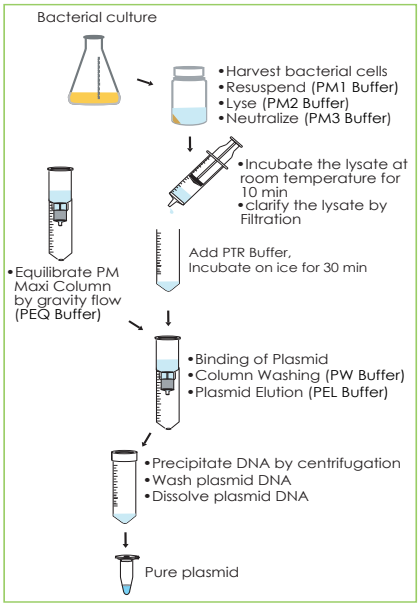
	DE-055EFs (2 preps_sample)	DE-055EF (10 preps)
PEQ Buffer	30 ml	135 ml
PM1 Buffer	42 ml	215 ml
PM2 Buffer	42 ml	215 ml
PM3 Buffer	42 ml	215 ml
PW Buffer	65 ml	270 ml+60 ml
PTR buffer	12 ml	55 ml
PEL Buffer	32 ml	215 ml
RNase A	4.2 mg	21.5 mg
Filter Maxi Cartridge	2 pcs	10 pcs
PM Maxi Column	2 pcs	10 pcs

Important Notes:

1. Store **RNase A powder** at -20 °C upon receipt of kit.
2. Add 0,5 ml of PM1 Buffer to a RNase A tube, vortex the tube to mix well.
   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. Pre-chill PM3 Buffer at 4 °C before starting.

Additional Requirements:

1. 50 ml tubes
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



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General Protocol:
Please Read Important Notes Before Starting Following Steps.

Harvest bacterial cells

1. Harvest the cells (up to 240 ml)by centrifugation at 4,500 ~ 6,000 x g at 4 °C for 10 min and discard the supernatant.

Equilibrate PM Maxi Column

2. Place a PM Maxi Column onto a 50 ml tube.

3. Equilibtate the PM Maxi column by applying 10 ml of PEQ Buffer. Allow the column to empty by gravity flow and discard the filtrate.

Cell lysis and lysate neutralization

4. Add 16 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.

5. Add 16 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 16 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 that the density of cultured cell is optimal the buffers volume (PM1, PM2, PM3) should be increased proportionally to culture volume.

(example): Culture Volume: 120-240 ml : PM1 16 ml, PM2 16 ml, PM3 16 ml

Culture Volume: 240-480 ml : PM1 32 ml, PM2 32 ml, PM3 32 ml

Make sure cell pellet be suspended completely within Buffer PM1

Mix the sample mixture completely after adding Buffer PM2 and Buffer PM3

Lysate filtration, endotoxin removal

8. Pour the lysate into FILTER cartridge barrel. Incubate the lysate for 10 minutes at room temperature to make the precipitate float up.

Note! To ensures filtration without clogging,10 minutes incubation is essential to make the precipitate float up.

9. Remove the cap from the tip of the FILTER CARTRIDGE Gently insert the plunger into Filter cartridge and filter the lysate into a clean 50 ml tube (PM Maxi Column) then allow it to flow through by gravity flow.

10.Add 5 ml o PTR u er to the iltrate and mi gentl pipetting incu ate it on ice or 0 min. A ter the incu ation the sample mi ture will ecome clear

Binding of Plasmid:

11. Transfer the half of the filtrate from step 10 to the equilibrated PM Maxi column. Allow the PM Maxi Column to empty by gravity flow and discard the flow-through.

12. Repeat step 11 for the rest of the filtrate.

Wash PM Maxi Columns

13. Wash PM Maxi Column by applying 30 ml of PW Buffer. Allow the PW Buffer to flow through the PM Maxi columnby gravity flow and discard the filtrate

Elution

14. Place the PM Maxi column onto a clean 50 ml centrifuge tube (not provided). Add 15 ml of PEL Buffer to elute DNA by gravity flow.

Precipitate plasmid DNA

15.Transfer the eluate from step 14 to a centrifuge tube. Add 0,75 volume of room temperature isopropanol to the eluated and mix well by inverting the tube 10 times.(ex: 11,25 ml isopropanol to 15 ml eluate)

Note! Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.

16. Centrifuge the tube at 5,000 x g at 4 °C for 30 min. Preferible centrifuge the tube at 15,000-20,000 xg

Wash and dissolve plasmid DNA

17.Carefully remove the supernatant and wash the plasmid pellet with 5 ml of room temperature 70% ethanol.

18. Centrifuge the tube at ≥ 5,000 x g at 4 °C for 10 min.

19. 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.)

20. 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 complelety 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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