DNA-maxi™ Endotoxin-Free Plasmid DNA Purification Kit

For Plasmid DNA Maxi prep.

RUO *Research Use Only* REF 17258 Σ 10 4℃ 25℃

DESCRIPTION

The endotoxins are released from cells in a small amounts during cell growth and in very large quantities upon cell death and lysis. Like undamaged cells, the LPS molecules induce inflammatory responses in the mammalian immune system. Therefore, they must be quantitatively removed from plasmid DNA preparations to guarantee high transfection rates and high viability of transfected cells. Due to their amphiphilic character and their negative charge endotoxins behave like DNA and are co-purified with most common plasmid purification systems. Regular silica membrane kits with a purification procedure based on chaotropic salt lead to plasmid DNA with an endotoxin level of > 1000 EU/µg. Anion exchange kits like DNA-maxi™ Endotoxin-Free Plasmid DNA Purification Kit reduce endotoxins to a level of < 0.1 EU/µg. This can be highly efficient for transfection of very sensitive cells like primary or neuronal cells.

KIT CONTENTS

Components	10 prep
M1 Buffer (Resuspension buffer)[1]	215 ml
M2 Buffer (Lysis buffer)[2]	215 ml
M3 Buffer (Neutralization buffer)	215 ml
EQ(Equilibration) Buffer	135 ml
ER(Endotoxin Remove) Buffer	55 ml
Washing Buffer	270 ml + 60 ml
Elution Buffer	215 ml
RNase A[3]	21.5 mg
Maxi Column	10 pcs
Storage Conditions : Room Temperature	

[1] Briefly spin the dissolved RNase A solution and add the RNase A solution to M1 Buffer. Before use, store M1 Buffer at 4℃ after adding RNase A solution.

[2] Check M2 Buffer for SDS precipitation due to low storage temperature in which case it is necessary to dissolve the SDS by warming at 37℃.

[3] RNase A can be stored at room temperature (15-25℃) for one year.

SPECIFICATION

- Technology : Anion-exchange chromatography (gravity-flow column)
- Lysate clarification : Centrifugation
- 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

IMPORTANT NOTES

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of M1 Buffer to a RNase A tube and vortex the tube to mix well. Transfer the total RNase A mixture back to the M1 bottle and mix well by vortexing. Store the M1 buffer at 4 °C.
3. If precipitates have formed in M2 Buffer, warm the buffer in 37°C waterbath to dissolve preciptates.
4. Pre-chill M3 Buffer at 4 °C before starting.

ADDITIONAL REQUIREMENTS

1. 50ml 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 ddH_2O

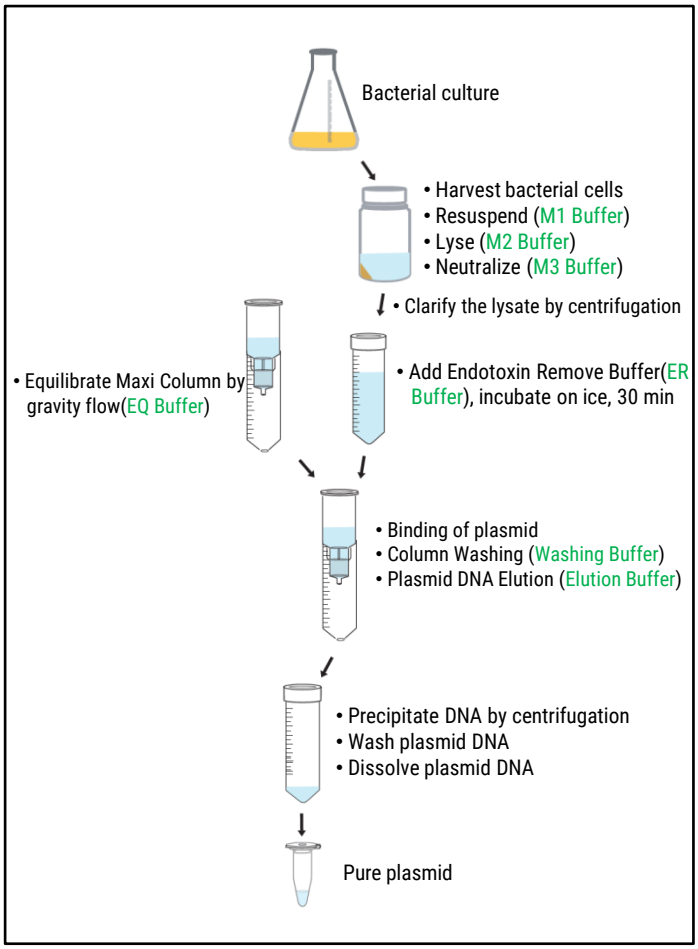
PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our CRT center is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If a iNtRON product does not meet your expectations, simply call your local distributor. If you have questions about product specifications or performance, please call iNtRON Technical Services or your local distributor.

NOTICE BEFORE USE

The DNA-maxi™ Endotoxin-Free Plasmid DNA Purification Kit is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of disease. All due care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general labolatory precaution and utilize safety while using this kit.

DIAGRAM OF EXPERIMENT



APPLICATION

Plasmid DNA isolated using the DNA-maxi™ Endotoxin-Free Plasmid DNA Purification Kit is suitable for use in a variety of downstream applications including:

- ✓ Mammalian cell transfection
- ✓ in vivio transfection
- ✓ in vitro transcription
- ✓ Protein expression
- ✓ DNA sequencing
- ✓ PCR cloning

PROTOCOL

1. **Harvest the cells by centrifugation at 4,500 ~ 6,000 x g at 4 °C for 10 min and discard the supernatant.**
2. **Place a Maxi Column onto a 50 ml tube.**
3. **Equilibrate the Maxi column by applying 10 ml of EQ Buffer. Allow the column to empty by gravity flow and discard the filtrate.**
4. **Add 16 ml of M1 Buffer(RNase A added) to resuspend the cell pellet by vortexing or pipetting.**
5. **Add 16 ml of M2 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 M3 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 (M1, M2, M3) should be increased proportionally to the culture volume. (ex. culture volume, 120 ~ 240 ml ; M1, M2, M3 = 16 ml / culture volume, 240 ~ 480 ml ; M1, M2, M3 = 32 ml) Make sure cell pellet be suspended completely within Buffer M1. Mix the sample mixture completely after adding Buffer M2 and Buffer M3.

8. **Centrifuge the tube at ≥ 5,000 x g at 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 5 ml of ER Buffer and mix gently by pipetting. Incubate the sample mixture on ice for 30 minutes. After the incubation, the sample mixture will become clear.**
11. **Transfer the half of the sample mixture from step 10 to the equilibrated Maxi Column. Allow sample mixture to flow through the Maxi Column by gravity flow and discard the filtrate.**
12. **Repeat step 11 for the rest of the sample mixture.**
13. **Wash the Maxi column by applying 30 ml of Washing Buffer. Allow Washing Buffer to flow through the Maxi Column by gravity flow and discard the filtrate.**
14. **Place the Maxi column onto a clean 50 ml centrifuge tube (not provided). Add 15 ml of Elution Buffer to the Maxi Column to elute the plasmid by gravity flow.**
15. **Transfer the eluate from step 14 to a centrifuge tube. Add 0.75 volume of room temperature isopropanol to the eluate and mix well by inverting the tube 10 times. (Ex : add 11.25 ml isopropanol to 15 ml eluate)**
16. **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.**

NOTE : Make sure that isopropanol be mixed thoroughly with eluate before centrifugation.

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.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Low yield	
1) Bacterial cells were not lysed completely	• After M3 Buffer addition, break up the precipitate by inverting.
2) Too many bacterial cells were used.	• By adjusting the incubation time, reduce the number of cells.
3) DNA failed to precipitate or DNA pellet was lost after precipitation.	• Measure correctly the volume of eluate in each centrifugation tube and add exactly 0.75 volume of isopropanol. • Centrifuge plasmid DNA at the appropriate speed, time, and temperature.
4) DNA pellet was insufficiently redissolved.	• Make sure that a sufficient amount volume(≥ 300ul) of Elution Buffer is added.
Purified DNA dose not perform well in downstream application	
1) RNA contamination	• Make sure that RNase A was added in M1 Buffer when first using. If RNase A added M1 Buffer is overdue, add additional RNase A. • Too many bacterial cells were used, reduce the sample volume.
2) Genomic DNA contamination	• Do not use overgrown bacterial culture. • During M2 and M3 Buffer addition, mix gently to prevent genomic DNA shearing. • Lysis time was too long (over 5 minutes).
3) Too much salt residual in DNA pellet	• Wash the DNA pellet twice with 70% ethanol.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
MacCell™ DH5α (10^7)	1 ㎖ (50 ㎕ x 20 vials)	15052
MacCell™ DH5α (10^8)	1 ㎖ (50 ㎕ x 20 vials)	15053
MacCell™ DH5α (10^9)	1 ㎖ (50 ㎕ x 20 vials)	15054
Fast DNA-spin™ Plasmid DNA Purification Kit	50 / 200 col.	17095 / 17013
DNA-spin™ Plasmid DNA Purification Kit	50 / 200 col.	17096 / 17098
DNA-midi™ SV Plasmid DNA Purification Kit	25 col.	17252
DNA-midi™ GT Plasmid DNA Purification Kit	25 col.	17254
DNA-maxi™ SV Plasmid DNA Purification Kit	12 col.	17253
MEGAquick-spin™ Plus Total Fragment DNA Purification Kit	50 / 200 col.	17289 / 17290
α-Complementation Solution		15032

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Review date : 2018. 03. 29