Clean Plasmid TR DNA Kit

USER MANUAL

Manual revision v6.00

Catalog Numbers:
CPLT-D0096: 96 preps
CPLT-D0384: 384 preps

Batch No: See package

Shipping: room temperature

Storage and stability: CleanNA Particles PLT and RNase A should be stored at 4°C upon receipt, store all other components at room temperature. See page 3 for more storage information.

Intended use: Clean Plasmid TR is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Quality Control: Each lot of Clean Plasmid TR is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at info@cleanna.com or +31 (0) 182 22 33 50.

Safety precautions: When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.

Emergency: In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or info@cleanna.com.

Expiry: When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

FOR RESEARCH USE ONLY

Contents

Introduction and Principle

The CleanNA Plasmid TR DNA kit allows for the isolation of high pure Plasmid DNA from bacteria, suitable for use in transfection experiments.

Our CleanNA Plasmid TR DNA Kit combines the proprietary Transfection Ready buffer system with the convenience of our magnetic CleanNA Particles PLT. The system efficiently removes bacterial endotoxins during the isolation procedure, delivering high-quality endotoxin-free plasmid DNA for further downstream processing.

The use of our CleanNA Particles PLT makes the whole procedure suited for manual processing as well as automated processing on a liquid handling system (e.g. Dynamic Devices LYNX™, Hamilton STAR™). The scalability of the procedure offers the advantage of flexible, fast and efficient high throughput Plasmid DNA extraction for your lab, adding speed and consistency to your laboratory processes. With the Large Volume protocol, large batches of Endotoxin-free plasmid DNA can be isolated in a single batch procedure.

Yields vary according to plasmid copy number, E.coli strain, and conditions of growth. A 1 mL overnight culture in LB medium typically produces 10-15 µg of high copy plasmids.

The purified plasmid can be used directly for transfection, automated fluorescent DNA sequencing, such as with BigDye sequencing chemistry, as well as for other standard molecular biology techniques including PCR and restriction enzyme digestion.

Kit Contents and Materials

Kit Contents:

Product	CPLT-D0096	CPLT-D0384	Storage
Preps	1 x 96	4 x 96	n/a
PLT Lysis Buffer	30 mL	120 mL	15-25°C
PLT Wash Buffer 1	60 mL	250 mL	15-25°C
PLT Wash Buffer 2	66 mL	264 mL	15-25°C
PLT Wash Buffer 3	15 mL	60 mL	15-25°C
Elution Buffer	15 mL	60 mL	15-25°C
CleanNA Particles PLT	2.2 mL	8.8 mL	2-8°C
RNase A	50 µL	200 µL	2-8°C
PLT Binding Buffer	60 mL	250 mL	15-25°C
PLT Resuspension Buffer	30 mL	120 mL	15-25°C (once mixed with RNase A store at 2-8°C)
PLT Neutralization Buffer	15 mL	60 mL	15-25°C

Note: Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37°C and shaking gently.

Materials and Reagents to be supplied by User for clearance via centrifugation:

- Centrifuge capable of reaching at least 3,000-5,000 x g
- Rotor adapter for 96-well deep-well plates
- Magnetic separation device for 96-well deep-well plates (Recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50))
- 2.0 mL 96-well deep-well plates compatible with magnetic separation device (Recommend Nunc Part No. 278752)
- 2.0 mL 96-well deep-well plate for bacterial growth
- 8- or 12-channel pipette
- 100% ethanol
- Optional: Plate shaker (Recommend Eppendorf MixMate)

Materials and Reagents to be supplied by User for Large Volume Protocol:

- Clean Plasmid TR DNA Kit
- Centrifuge capable of at least 3,000-5,000 x g
- Rotor adapter for 50 mL centrifuge tubes
- Magnetic separation device compatible with 50 mL centrifuge tubes
- Clean 50 mL centrifuge tubes
- Suitable Erlenmeyer for bacterial growth
- Pipettes
- Serological pipettes
- 100% ethanol
- Vortex or shaker

Preparation of Reagents

PLT Wash Buffer 2
Dilute PLT Wash Buffer 2 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPLT-D0096	84 mL
CPLT-D0384	336 mL

PLT Wash Buffer 3
Dilute PLT Wash Buffer 3 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPLT-D0096	60 mL
CPLT-D0384	240 mL

PLT Resuspension Buffer
Add RNase A to the bottle of PLT Resuspension Buffer before use. Store at 2-8°C after RNase A addition.

Protocol for isolation of Endotoxin-free plasmid DNA

This protocol describes the procedure for the isolation of transfection ready, endotoxin-free Plasmid DNA from 1 up to 1,5 mL bacterial cultures.

Before Starting:

- Prepare the PLT Wash Buffer 2, PLT Wash Buffer 3 and PLT Resuspension Buffer according the instructions on page 4.

Protocol:

1. Culture Volume: Inoculate 1-1.5 mL LB/antibiotic(s) medium with E. coli in a 96-well deep-well plate and incubate at 37°C with agitation for 12-16 hours.

   Note: It is strongly recommended that an end A negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Centrifuge at 2,000-3,000 x g at room temperature for 10 minutes to pellet bacteria.
3. Discard the supernatant. Dry the plate by inverting the plate on a absorbent paper towel to remove excess media.
4. Add 250 µL PLT Resuspension Buffer to the bacterial pellet in each sample. Resuspend the cells completely by vortexing. Complete resuspension of the cell pellet is vital for obtaining good plasmid yields.

   Note: RNase A must be added to PLT Resuspension Buffer before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. 250 µL PLT Lysis Buffer. Gently mix by shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 5 minute incubation at room temperature may be necessary. Avoid vigorous mixing as doing so will shear chromosomal DNA and lowers plasmid purity. Store PLT Lysis Buffer tightly capped when not in use.
6. Add 125 µL PLT Neutralization Buffer. Mix by gently shaking the plate until a flocculent white precipitate forms.
7. Centrifuge at 2,000-3,000 x g at room temperature for 10 minutes.
8. Transfer 500 µL cleared cell lysate into a new 96-well deep-well plate (2.2 mL) not provided. Avoid transferring the white precipitate containing cell debris.
9. Add 500 µL PLT Binding Buffer and 20 µL CleanNA Particles PLT. Mix thoroughly by pipetting up and down 10 times.
10. Incubate for 5 minutes at room temperature.
11. Place the plate on the magnetic separation device to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.

    Note: Magnetization time depends on the magnet used and plasticware but should take ~3-5 minutes. If CleanNA Particles PLT are floating at top of surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom to move the CleanNA Particles PLT closer to the magnet.

12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
13. Remove the plate from the magnetic separation device.
14. Add 500 µL PLT Wash Buffer 1. Resuspend the particles by pipetting up and down 10 times or shaking for 1 minute at 1,300 RPM.

15. Place the plate on a magnetic separation device to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
17. Remove the plate from magnetic separation device.
18. Add 700 µL PLT Wash Buffer 2. Resuspend the particles by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.
19. Place the plate on the magnetic separation device to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
21. Remove the plate from magnetic separation device.
22. Repeat Steps 18-21 once for a second PLT Wash Buffer 2 wash step.
23. Add 700 µL PLT Wash Buffer 3. Resuspend the particles by pipetting up and down 10 times or shaking for 1 minute at 1,000 RPM.
24. Place the plate on a magnetic separation device to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
25. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT. Leave the plate on the magnetic separation device.
26. Incubate at room temperature for 10 minutes to dry the CleanNA Particles PLT.

    ⚠ **Note:** It is recommended to incubate for 1 minute then remove any remaining liquid from the wells then incubate for an additional 9 minutes.
27. Add 50-100 µL Elution Buffer heated to 65°C. Resuspend the particles by pipetting up and down for 20 times or by shaking at 1,500 RPM for 2-5 minutes.
28. Place the plate on a magnetic separation device to magnetize the CleanNA Particles PLT.
29. Transfer the cleared supernatant containing the purified plasmid into a new 96-well microplate (not provided). Seal the plate and store at -20°C.

Large volume protocol for isolation of Endotoxin-free plasmid DNA

This protocol has been adjusted for the isolation of transfection ready, endotoxin-free plasmid DNA from 30 mL of overnight culture.

Before Starting:

- Prepare the PLT Wash Buffer 2, PLT Wash Buffer 3 and PLT Resuspension Buffer according the instructions on page 4.

Protocol:

1. Culture Volume: Inoculate 30 mL LB/antibiotic(s) medium with E. coli in a Erlenmeyer flask and incubate at 37°C with agitation for 12-16 hours.

   Note: It is strongly recommended that an end A negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Transfer 30 mL of overnight culture into a 50 mL centrifuge tube (not provided).
3. Centrifuge at 2,000-3,000 x g at room temperature for 10 minutes to pellet bacteria.
4. Discard the supernatant. Dry the tube by carefully inverting the tube on a absorbent paper towel to remove excess media.
5. Add 5 mL PLT Resuspension Buffer to the bacterial pellet of each sample. Resuspend the cells completely by vortexing. Complete resuspension of the cell pellet is vital for obtaining good plasmid yields.
6. Add 5 mL of PLT Lysis Buffer. Gently mix by shaking and rotating the tube for 1 minute to obtain a cleared lysate. A 5 minute incubation at room temperature may be necessary. Avoid vigorous mixing as doing so will shear chromosomal DNA and lowers plasmid purity. Store PLT Lysis Buffer tightly capped when not in use.
7. Add 2,5 mL of PLT Neutralization Buffer. Mix by gently shaking the tube until a flocculent white precipitate forms.
8. Centrifuge at 2,000-3,000 x g at room temperature for 10 minutes.
9. Transfer 10 mL cleared cell lysate into a new 50 mL centrifuge tube (not provided). Avoid transferring the white precipitate containing cell debris.
10. Add 10 mL of PLT Binding Buffer and 400 µL CleanNA Particles PLT. Mix thoroughly by pipetting up and down 20 times or shaking for 2 minutes at 1,200 RPM until homogeneous.
11. Incubate for 5 minutes at room temperature.
12. Place the tube in the magnetic stand to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.

    Note: Magnetization time depends on the magnet used and plasticware but should take ~5-7 minutes. If CleanNA Particles PLT are floating at top of surface after 5 minutes, pipet from the top of the liquid and slowly dispense to the bottom to move the CleanNA Particles PLT closer to the magnet.

13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
14. Remove the tube from the magnetic separation device.
15. Add 10 mL PLT Wash Buffer 1. Resuspend the particles by pipetting up and down 20 times or shaking for 1 minute at 1,300 RPM.

16. Place the tube in the magnetic stand to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
17. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
18. Remove the tube from magnetic separation device.
19. Add 14 mL PLT Wash Buffer 2. Resuspend the particles by pipetting up and down 15 times or shaking for 1 minute at 1,000 RPM.
20. Place the tube in the magnetic stand to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT.
22. Remove the tube from magnetic separation device.
23. Repeat Steps 18-21 once for a second PLT Wash Buffer 2 wash step.
24. Add 14 mL PLT Wash Buffer 3. Resuspend the particles by pipetting up and down 15 times or shaking for 1 minute at 1,000 RPM.
25. Place the tube in the magnetic stand to magnetize the CleanNA Particles PLT. Incubate at room temperature until the CleanNA Particles PLT are completely cleared from the solution.
26. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles PLT. Leave the tube in the magnetic separation device.
27. Incubate at room temperature for 10 minutes to dry the CleanNA Particles PLT.

    

    Note: It is recommended to incubate for 1 minute then remove any remaining liquid from the tube(s) then incubate for an additional 9 minutes.
28. Add 1-2 mL Elution Buffer heated to 65°C. Resuspend the particles by pipetting up and down for 20 times or by shaking at 1,500 RPM for 2-5 minutes.
29. Place the tube in the magnetic stand to magnetize the CleanNA Particles PLT.
30. Transfer the cleared supernatant containing the purified plasmid into a new tube (not provided) for storage. Close the tube and store at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yield	Poor lysis of tissue.	Only use LB or YT. Do not use more than 1.2 mL.
		Cells may not be dispersed adequately prior to addition of PLT Lysis Buffer. Vortex cell suspension (after PLT Resuspension Buffer addition) to completely disperse.
		Increase incubation time with PLT Lysis Buffer to obtain a clear lysate.
		PLT Lysis Buffer, if not tightly closed, may need to be replaced.
	Bacterial clone is not fresh.	Use fresh glycerol cultures and avoid repeated freeze/thaw cycles of clones. Always make enough replica plates and use precultures for inoculation. The remainder of the precultures can be used to set up fresh glycerol stocks.
No DNA eluted	Lysate prepared incorrectly.	Check the stock of buffers and age of the buffers. Make sure the correct volume of buffer is added to the samples.
	Cells are not resuspended completely.	Pelleted cells should be completely resuspended with PLT Resuspension Buffer. Do not add PLT Lysis Buffer until an even cell suspension is obtained.

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Plasmid TR DNA Kit (1 x 96)	CPLT-D0096
Clean Plasmid TR DNA Kit (4 x 96)	CPLT-D0384

Product	Part Number
Clean Magnet Plate 96-Well	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
6.00	10/OCT/2023	Kit Contents and Materials	Text correction
5.00	October 2021	Total revision	Language and layout modifications
		Ordering information	Updated
4.00	September 2020	Total revision	New lay-out
			Important general user information added at page 1 (before contents)
		Large volume protocol for isolation of Endotoxin-free plasmid DNA	Protocol added