Clean Circulating LV DNA Kit

Instructions for Use

V.6 - APRIL 2024

For Research Use Only

REF **CCLV-D0004, CCLV-D0096**

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Contents

Intended Use

Clean Circulating LV 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.

Introduction and Principle

The Clean Circulating LV DNA Kit allows for the isolation of cell free DNA from sample volumes up to 4 mL plasma or serum. The entire procedure allows for both manual as well as automated sample processing.

The Clean Circulating LV DNA Kit combines our proprietary buffer system with the convenience of our magnetic CleanNA Particles LV, thereby eliminating the need for vacuum steps or funnels throughout the procedure. As a result, the Clean Circulating LV DNA Kit provides a procedure that can be used in automated protocols via a simple 4 step process (lyse, bind, wash and elute).

Our CleanNA Particles LV offer a high binding capacity and, combined with the buffer system, target smaller DNA fragments (120-400 bp). This combination minimizes the risk of genomic DNA contamination. The high binding capacity of the CleanNA Particles LV decrease the amount of particles required during binding steps thereby reducing the elution volume. This enables isolated cell free DNA from 4 mL plasma to be eluted in as little as 50 µL.

The protocol is scalable due to the use of our magnetic bead purification technology and can, besides manual usage, easily be automated on liquid handling workstations (Dynamic Devices LYNX™, Hamilton STAR™).

The isolated Cell Free DNA is ready for use in downstream applications such as Next Generation Sequencing (NGS) and (q)PCR.

Schematic Overview

The uniquely formulated lysis buffer releases the circulating DNA from proteins and vesicles bound to the DNA while DNases and RNases are inactivated. DNA is isolated from the lysate in one step by binding to CleanNA Particles' surface. The CleanNA magnetic particles are separated from the lysate by using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants, the purified DNA is eluted from the CleanNA particles using an Elution Buffer.

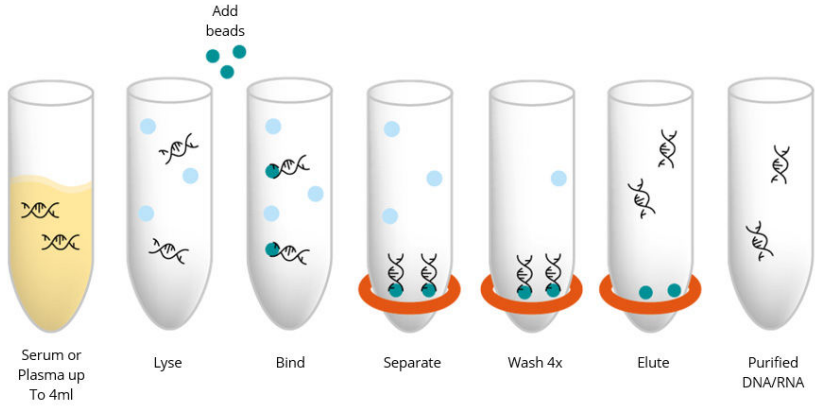

Figure 1: Schematic overview of the Clean Circulating LV DNA Kit extraction procedure.

Materials Provided

Kit Contents:

Component	CCLV-D0004 (4 preps)	CCLV-D0096 (96 preps)
CleanNA Particles LV	0,2 mL	4 mL
NLB Buffer	1,5 mL	30 mL
NSF Buffer	20 mL	450 mL
SF Wash	10 mL	2 x 120 mL
CG Wash	2,5 mL	2 x 20 mL
Elution Buffer	15 mL	250 mL
Proteinase K (20 mg/mL)	0,3 mL	7 mL

Reagent Shipping, Storage and Handling

Clean Circulating LV DNA Kit is shipped at room temperature (15-25 °C). Do not freeze the components of the Clean Circulating LV DNA Kit. After the components have been frozen, the kit is no longer suitable for use. Do not use the Clean Circulating LV DNA Kit after the expiration date stated on the outer box label.

Component	Storage Temperature
CleanNA Particles LV	2-8 °C
NLB Buffer	15-25 °C
NSF Buffer	15-25 °C
SF Wash	15-25 °C
CG Wash	15-25 °C
Elution Buffer	15-25 °C
Proteinase K (20 mg/mL)	15-25 °C (for storage > 12 months, store at 2-8 °C)

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

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

The LOT number on the CleanNA Particles LV box packaging is different from the LOT number on the CleanNA Particles LV bottle. The LOT number on the box matches the LOT number of the whole kit and the one on the bottle is specifically for the particles. Since the CleanNA Particles LV is stored at a different temperature than the rest of the kit, please make sure that the LOT number on the box packaging of the particles matches the LOT number of the kit before use.

Precautions

When working with chemicals, always follow your facility's procedures and universal precautions by using disposable gloves, safety glasses, a labcoat etc.

For all safety information, please consult the safety data sheet (SDS).

NLB Buffer	
	Causes serious eye damage. Causes skin irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider. IF ON SKIN: Wash with plenty of water and soap. If skin irritation occurs: Get medical advice/ attention. Take off contaminated clothing and wash it before reuse.
NSF Buffer	
	Flammable liquid and vapour. Causes serious eye damage. Harmful if swallowed. Causes skin irritation. Harmful to aquatic life with long lasting effects. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider. In case of fire: Use alcohol resistant foam or normal protein foam to extinguish. F SWALLOWED: Call a POISON CENTER/doctor/physician/first aider if you feel unwell. IF ON SKIN: Wash with plenty of water and soap. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. Rinse mouth. If skin irritation occurs: Get medical advice/attention. Take off contaminated clothing and wash it before reuse.
Proteinase K (20 mg/mL)	
	May cause allergy or asthma symptoms or breathing difficulties if inhaled. **IF INHALED:** Remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a POISON CENTER/doctor/physician/first aider.
SF Wash	
	Causes severe skin burns and eye damage. Harmful if swallowed. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider. Wash contaminated clothing before reuse. IF SWALLOWED: Call a POISON CENTER/doctor/physician/first aider if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing.

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of Clean Circulating LV DNA Kit according to predetermined and validated protocols in the Quality Management System (QMS). Additionally, a quality check after production of each lot is performed to secure consistent product quality. CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

For isolation in single tubes

Materials and Reagents to be supplied by user for the Tube Protocol for up to 1 mL of sample input:

- 100% ethanol
- Magnetic separation device for 1.5/2.0 mL tubes
- Vortexer
- Shaker or Rocker
- Incubator capable to be set at 60°C
- 1.5 mL micro centrifuge tube(s)
- 15 mL centrifuge tube(s)

Materials and Reagents to be supplied by user for the Tube Protocol for up to 2 mL and up to 4 mL of sample input:

- 100% ethanol
- Magnetic separation device for 15 mL centrifuge tubes
- Magnetic separation device for 1.5 / 2.0 mL tubes
- Vortexer
- Shaker or Rocker
- Incubator capable to be set at 60°C
- 1.5 mL micro centrifuge tube(s)
- 15 mL centrifuge tube(s), compatible with magnetic separation device used

For isolation using 96-well plate format

Materials and Reagents to be supplied by user for the Plate Protocol for up to 1 mL of sample input:

- 100% ethanol
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- Shaker or Rocker
- Incubator capable to be set at 60°C
- 24-well deep-well plate(s), 10 mL (Cat# Whatman 7701-5102)
- 96-well deep-well plate(s)

Materials and Reagents to be supplied by user for the Plate Protocol for up to 2 mL and up to 4 mL of sample input:

- 100% ethanol
- Magnetic separation device for 24-well plates
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- Shaker or Rocker
- Incubator capable to be set at 60°C
- 24-well deep-well plate(s), 10 mL (Cat# Whatman 7701-5102)
- 96-well deep-well plate(s)

Preparation of Reagents

CG Wash

Prepare CG Wash with absolute ethanol as follows and store at room temperature.

Kit	Absolute ethanol to be added
CCLV-D0004	10 mL
CCLV-D0096	80 mL

Tube protocol for up to 1 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.
• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.
• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 1 mL plasma/serum samples to a 15 mL centrifuge tube (not provided).
2. Bring the sample volume up to 1 mL with Elution Buffer (provided with this kit) if the sample volume is less than 1 mL.
3. Add 15 µL Proteinase K.
4. Add 67 µL NLB Buffer.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain the most efficient DNA binding to the CleanNA Particles LV.

8. Add 1 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 10 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Transfer 1 mL lysate to a 1.5 mL micro centrifuge tube (not provided).
12. Place the tube on a magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Transfer the remaining lysate from step 11 to the 1.5 mL micro centrifuge tube

used in the previous steps.

15. Place the tube on a magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
17. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
18. Add 500 µL SF Wash.
19. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

20. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
22. Repeat steps 17-21 for a second “SF Wash” wash step.
23. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
24. Add 500 µL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

25. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.
26. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
28. Repeat Steps 23-27 for a second “CG Wash” wash step.
29. Remove the tube from the magnetic separation device for approximately 30 seconds.
30. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV.
31. Aspirate and discard the residual CG Wash.
32. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
33. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.

34. Add 30-60 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
35. Incubate at room temperature for 5 minutes, while constantly vortexing.
36. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
37. Transfer the cleared supernatant containing purified DNA to a clean 1.5 mL micro centrifuge tube (not provided).
38. Store DNA at -20°C.

Tube protocol for up to 2 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.
• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.
• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 2 mL plasma/serum samples to a 15 mL centrifuge tube (not provided). Choose the correct plastic ware depending on the magnetic separation device being used.
2. Bring the sample volume up to 2 mL with Elution Buffer (provided with this kit) if the sample volume is less than 2 mL.
3. Add 30 µL Proteinase K.
4. Add 135 µL NLB Buffer.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 25 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain most efficient DNA binding to the CleanNA Particles LV.

8. Add 2 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 20 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Place the tube on a magnetic separation device, compatible with the 15 mL tube, to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
13. Remove the tube/plate containing the CleanNA Particles LV from the magnetic

separation device.

14. Add 1 mL SF Wash.
15. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

16. Transfer the resuspended CleanNA Particles LV to a new 1.5 mL microcentrifuge tube (not provided).

⚠ **Note:** Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure.

17. Place the 1.5 mL tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
18. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
19. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
20. Add 1 mL SF Wash.
21. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

22. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
24. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
25. Add 1 mL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

26. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.
27. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
29. Repeat Steps 24-28 for a second CG Wash wash step.
30. Remove the tube from the magnetic separation device for approximately 30

seconds.

31. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV.
32. Aspirate and discard the residual CG Wash.
33. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
34. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
35. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
36. Incubate at room temperature for 5 minutes, while constantly vortexing.
37. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
38. Transfer the cleared supernatant containing purified DNA to a clean 1.5 mL micro centrifuge tube (not provided).
39. Store DNA at -20°C.

Tube protocol for up to 4 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.
• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.
• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 4 mL plasma/serum samples to a 15 mL centrifuge tube (not provided). Choose the correct plastic ware depending on the magnetic separation device being used.
2. Bring the volume up to 4 mL with Elution Buffer (provided with this kit) if the sample volume is less than 4 mL.
3. Add 60 µL Proteinase K.
4. Add 270 µL NLB Buffer.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain most efficient DNA binding to the CleanNA Particles LV.

8. Add 4 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 30 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Place the tube on a magnetic separation device, compatible with the 15 mL tube, to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
13. Remove the tube containing the CleanNA Particles LV from the magnetic separation

device.

14. Add 1 mL SF Wash.
15. Resuspend the CleanNA Particles LV by vortexing for 5 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

16. Transfer the resuspended CleanNA Particles LV to a new 1.5 mL micro centrifuge tube (not provided).

⚠ **Note:** Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure.

17. Place the 1.5 mL tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
18. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
19. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
20. Add 1 mL SF Wash.
21. Resuspend the CleanNA Particles LV by vortexing for 5 minute or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

22. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
24. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
25. Add 1 mL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

26. Resuspend the CleanNA Particles LV by vortexing for 5 minutes or pipetting up and down 20 times.
27. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
29. Repeat Steps 24-28 for a second CG Wash wash step.
30. Remove the tube from the magnetic separation device for approximately 30

seconds.

31. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV.
32. Aspirate and discard the residual CG Wash.
33. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
34. Remove the tube containing the CleanNA Particles LV from the magnetic separation device.
35. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
36. Incubate at room temperature for 5 minutes, while constantly vortexing.
37. Place the tube on the magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
38. Transfer the cleared supernatant containing purified DNA to a clean microplate or 1.5 mL micro centrifuge tube (not provided).
39. Store DNA at -20°C.

Plate protocol for up to 1 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.

• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.

• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 1 mL plasma/serum samples to a 24-well Deep Well Plate (not provided).
2. Bring the sample volume up to 1 mL with Elution Buffer (provided with this kit) if the sample volume is less than 1 mL.
3. Add 15 µL Proteinase K.
4. Add 67 µL NLB Buffer, optionally seal the plate.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain most efficient DNA binding to the CleanNA Particles LV.

8. Add 1 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 10 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the 24-well plate containing the CleanNA Particles LV from the magnetic

separation device.

15. Add 500 µL SF Wash.
16. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

17. Transfer the resuspended CleanNA Particles LV to a new 96-well Deep Well Plate (not provided).

⚠ **Note:** Continue to work in 96-well format for the remaining procedure.

18. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
19. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
21. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
22. Add 500 µL SF Wash.
23. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
25. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
26. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
27. Add 500 µL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

28. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.
29. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
30. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
31. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
32. Repeat Steps 26-31 for a second “CG Wash” wash step.

33. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
34. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
35. Aspirate and discard the residual CG Wash.
36. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
37. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
38. Add 30-60 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
39. Incubate at room temperature for 5 minutes, while constantly mixing by pipetting, shaking or vortexing.
40. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
41. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
42. Transfer the cleared supernatant containing purified DNA to a clean 96-well plate of to clean individual tubes (not provided).
43. Store DNA at -20°C.

Plate protocol for up to 2 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.
• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.
• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 2 mL plasma/serum samples to a 24-well Deep Well Plate (not provided).
2. Bring the sample volume up to 2 mL with Elution Buffer (provided with this kit) if the sample volume is less than 2 mL.
3. Add 30 µL Proteinase K.
4. Add 135 µL NLB Buffer, optionally seal the plate.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 25 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain most efficient DNA binding to the CleanNA Particles LV.

8. Add 2 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 20 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the 24-well plate containing the CleanNA Particles LV from the magnetic

separation device.

15. Add 1 mL SF Wash.
16. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

17. Transfer the resuspended CleanNA Particles LV to a new 96-well Deep Well Plate (not provided).

⚠ **Note:** Continue to work in 96-well format for the remaining procedure.

18. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
19. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
21. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
22. Add 1 mL SF Wash.
23. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
25. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
26. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
27. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
28. Add 1 mL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

29. Resuspend the CleanNA Particles LV by vortexing for 2 minutes or pipetting up and down 20 times.
30. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
31. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
32. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.

33. Repeat Steps 26-31 for a second “CG Wash” wash step.
34. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
35. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
36. Aspirate and discard the residual CG Wash.
37. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
38. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
39. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
40. Incubate at room temperature for 5 minutes, while constantly mixing by pipetting, shaking or vortexing.
41. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
42. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
43. Transfer the cleared supernatant containing purified DNA to a clean 96-well plate of to clean individual tubes (not provided).
44. Store DNA at -20°C.

Plate protocol for up to 4 mL Serum/Plasma

Before Starting:

• Set incubator to 60°C.
• Prepare CG Wash according to the instructions in the Preparation of Reagents section on Page 11.
• Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

Protocol:

1. Add up to 4 mL plasma/serum samples to a 24-well Deep Well Plate (not provided).
2. Bring the sample volume up to 4 mL with Elution Buffer (provided with this kit) if the sample volume is less than 4 mL.
3. Add 60 µL Proteinase K.
4. Add 270 µL NLB Buffer, optionally seal the plate.
5. Vortex at maximum speed or pipet up and down to mix thoroughly.
6. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.
7. Incubate at room temperature for 10 minutes.

⚠ **Note:** This incubation step is crucial to let the sample temperature drop and obtain most efficient DNA binding to the CleanNA Particles LV.

8. Add 4 mL NSF Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 30 µL CleanNA Particles LV. Invert the sample 10 times or pipet up and down to mix.
10. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking.

⚠ **Note:** Do not vortex at high speeds as this will cause foaming resulting in a reduced yield. The speed of mixing should be set to continuously keep the CleanNA Particles LV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the 24-well plate containing the CleanNA Particles LV from the magnetic

separation device.

15. Add 1 mL SF Wash.
16. Resuspend the CleanNA Particles LV by vortexing for 5 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

17. Transfer the resuspended CleanNA Particles LV to a new 96-well Deep Well Plate (not provided).

⚠ **Note:** Continue to work in 96-well format for the remaining procedure.

18. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
19. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
21. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
22. Add 1 mL SF Wash.
23. Resuspend the CleanNA Particles LV by vortexing for 5 minutes or pipetting up and down 20 times.

⚠ **Note:** To obtain good purity, complete resuspension of the CleanNA Particles LV is critical.

24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
25. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
26. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
27. Add 1 mL CG Wash.

⚠ **Note:** CG Wash must be diluted with 100% ethanol prior to use. Please see Page 11 for instructions.

28. Resuspend the CleanNA Particles LV by vortexing for 5 minutes or pipetting up and down 20 times.
29. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
30. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
31. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
32. Repeat Steps 26-31 for a second “CG Wash” wash step.

33. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
34. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
35. Aspirate and discard the residual CG Wash.
36. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles LV.
37. Remove the 96-well plate containing the CleanNA Particles LV from the magnetic separation device.
38. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles LV by vortexing or pipetting up and down 20 times.
39. Incubate at room temperature for 5 minutes, while constantly mixing by pipetting, shaking or vortexing.
40. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles LV.
41. Incubate at room temperature until the CleanNA Particles LV are completely cleared from solution.
42. Transfer the cleared supernatant containing purified DNA to a clean 96-well plate of to clean individual tubes (not provided).
43. Store DNA 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	Suggestion
Low DNA yield	Incomplete resuspension of CleanNA Particles LV	Resuspend the CleanNA Particles LV by vortexing vigorously before use.
	Inefficient binding of the DNA to the CleanNA Particles LV	Ensure to let the sample cool at room temperature for 10 minutes prior to addition of the NSF Buffer.
	Inefficient binding of the DNA to the CleanNA Particles LV	Ensure to mix each sample continuously throughout the binding incubation.
	Loss of CleanNA Particles LV during operation.	Avoid disturbing the CleanNA Particles LV during aspiration.
	DNA remains bound to CleanNA Particles LV.	Increase elution volume and incubate at room temperature for 15 minutes; Pipet up and down 50 to 100 times.
	DNA washed off.	Dilute CG Wash by adding appropriate volume of 100% ethanol prior to use (see Page 11 for instructions).
	Ethanol carryover.	Dry the CleanNA Particles LV at room temperature for 25 minutes before elution.
CleanNA Particles LV do not completely clear from solution	Too short magnetizing time.	Increase collection time on the magnetic separation device.
High Molecular Weight Co- Purification	Two SF Wash Steps must be performed.	Perform two SF Wash steps as instructed in the manual. Increase the volume of wash buffer if necessary.
Problems in downstream applications	Salt carryover.	CG Wash must be at room temperature.
Abnormal BioAnalyzer data	BioAnalyzer shows multiple sharp peaks during analysis.	Ensure to remove all traces of the cleared supernatant after each wash step.
		Ensure to incubate the tube/plate for 25 minutes to dry the CleanNA Particles LV.
	BioAnalyzer shows base line climbing towards the end.	Check the BioAnalyzer chip for air bubbles. Load samples onto a new freshly prepared chip.
	BioAnalyzer shows high blob at the beginning of the trace.	Ensure the purified sample does not contain traces of CleanNA Particles LV.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Circulating LV DNA Kit (4 preps)	CCLV-D0004
Clean Circulating LV DNA Kit (96 preps)	CCLV-D0096

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

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
6.00	18/APR/2024	Total revision	Changed layout according to current CleanNA corporate style.
			Updated buffer names.
5.00	October 2021	Total revision	Language and layout revisions.
		All protocols	Clarified mixing step for elution incubation step.
		Materials and reagents to be supplied by user	Added 24-well magnet plate to user requirements for 2 up to 4 mL serum/plasma plate protocols.
4.00	August 2020	Total revision	New layout.
			Important general information added at page 1 (before contents).

Notes

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