Clean Circulating LV DNA Kit

USER MANUAL

Manual revision v5.00

Catalog Numbers:
CCLV-D0004: 4 preps
CCLV-D0096: 96 preps
CCLV-D0384: 384 preps

Batch No: See package

Shipping: Room temperature

Storage and stability: CleanNA Particles CCLV 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 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.

Quality Control: Each lot of Clean Circulating LV 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

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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 CCLV particles, 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).

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.

Our CleanNA Particles CCLV 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 CCLV 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.

Kit Contents and Materials

Kit Contents:

Product	CCLV-D0004	CCLV-D0096	CCLV-D0384	Storage
Preps	4	96	384	n/a
CleanNA Particles CCLV	200 µL	4 mL	13 mL	2-8°C
CCLV Lysis	1.5 mL	30 mL	120 mL	20-25°C
CCLV Binding	20 mL	450 mL	2 x 850 mL	20-25°C
CCLV Wash 1	10 mL	2 x 120 mL	900 mL	20-25°C
CCLV Wash 2	2.5 mL	3 x 20 mL	200 mL	20-25°C
Elution Buffer	10 mL	250 mL	1000 mL	20-25°C
Proteinase K Solution	300 µL	7 mL	28 mL	20-25°C (for storage > 12 months, store at 2-8°C)

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

Wash 2

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

Kit	100% Ethanol to be Added
CCLV-D0004	10 mL
CCLV-D0096	80 mL
CCLV-D0384	800 mL

Tube protocol for up to 1 mL Serum/Plasma

Before Starting:

- Set incubator to 60°C.
- Prepare CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 67 µL CCLV Lysis.
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 CCLV.

8. Add 1 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 10 µL CleanNA Particles CCLV. 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 CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
17. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
18. Add 500 µL CCLV Wash 1.

19. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

20. Place the tube on the magnetic separation device to magnetize the CleanNA Particles CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
22. Repeat steps 17-21 for a second "CCLV Wash 1" wash step.
23. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
24. Add 500 µL CCLV Wash 2.

Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

25. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
28. Repeat Steps 23-27 for a second "CCLV Wash 2" 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 CCLV.
31. Aspirate and discard the residual CCLV Wash 2.
32. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
33. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
34. Add 30-60 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV 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 CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 135 µL CCLV Lysis.
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 CCLV.

8. Add 2 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 20 µL CleanNA Particles CCLV. 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 CCLV resuspended in solution.

11. Place the tube on a magnetic separation device, compatible with the 15 mL tube, to magnetize the CleanNA Particles CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
13. Remove the tube/plate containing the CleanNA Particles CCLV from the magnetic separation device.
14. Add 1 mL CCLV Wash 1.
15. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

16. Transfer the resuspended CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
18. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
19. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
20. Add 1 mL CCLV Wash 1.
21. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

22. Place the tube on the magnetic separation device to magnetize the CleanNA Particles CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
24. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
25. Add 1 mL CCLV Wash 2.

Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

26. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
29. Repeat Steps 24-28 for a second CCLV Wash 2 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 CCLV.
32. Aspirate and discard the residual CCLV Wash 2.
33. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
34. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
35. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV 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 CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 270 µL CCLV Lysis.
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 CCLV.

8. Add 4 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 30 µL CleanNA Particles CCLV. 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 CCLV resuspended in solution.

11. Place the tube on a magnetic separation device, compatible with the 15 mL tube, to magnetize the CleanNA Particles CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
13. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
14. Add 1 mL CCLV Wash 1.
15. Resuspend the CleanNA Particles CCLV by vortexing for 5 minutes or pipetting up and down 20 times.

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

16. Transfer the resuspended CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared

from solution.

18. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
19. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
20. Add 1 mL CCLV Wash 1.
21. Resuspend the CleanNA Particles CCLV by vortexing for 5 minute or pipetting up and down 20 times.

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

22. Place the tube on the magnetic separation device to magnetize the CleanNA Particles CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
24. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
25. Add 1 mL CCLV Wash 2.

Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

26. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
29. Repeat Steps 24-28 for a second CCLV Wash 2 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 CCLV.
32. Aspirate and discard the residual CCLV Wash 2.
33. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
34. Remove the tube containing the CleanNA Particles CCLV from the magnetic separation device.
35. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV. Incubate at room temperature until the CleanNA Particles CCLV 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 CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 67 µL CCLV Lysis, 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 CCLV.

8. Add 1 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 10 µL CleanNA Particles CCLV. 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 CCLV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
14. Remove the 24-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
15. Add 500 µL CCLV Wash 1.
16. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

17. Transfer the resuspended CleanNA Particles CCLV 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 CCLV.
19. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
21. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
22. Add 500 µL CCLV Wash 1.
23. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

    Note: To obtain good purity, complete resuspension of the CleanNA Particles CCLV is critical.
24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
25. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.

    Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
26. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
27. Add 500 µL CCLV Wash 2.

    Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.
28. Resuspend the CleanNA Particles CCLV 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 CCLV.
30. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
31. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
32. Repeat Steps 26-31 for a second “CCLV Wash 2” wash step.
33. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
34. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
35. Aspirate and discard the residual CCLV Wash 2.
36. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
37. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
38. Add 30-60 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV.
41. Incubate at room temperature until the CleanNA Particles CCLV 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 CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 135 µL CCLV Lysis, 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 CCLV.

8. Add 2 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 20 µL CleanNA Particles CCLV. 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 CCLV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
14. Remove the 24-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
15. Add 1 mL CCLV Wash 1.
16. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

17. Transfer the resuspended CleanNA Particles CCLV 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 CCLV.
19. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
21. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
22. Add 1 mL CCLV Wash 1.
23. Resuspend the CleanNA Particles CCLV by vortexing for 2 minutes or pipetting up and down 20 times.

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

24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
25. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
26. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
27. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
28. Add 1 mL CCLV Wash 2.

Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

29. Resuspend the CleanNA Particles CCLV 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 CCLV.
31. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
32. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
33. Repeat Steps 26-31 for a second “CCLV Wash 2” wash step.
34. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
35. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
36. Aspirate and discard the residual CCLV Wash 2.
37. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
38. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
39. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV.
42. Incubate at room temperature until the CleanNA Particles CCLV 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 CCLV Wash 2 according to the instructions in the Preparing Reagents section on Page 4.
- Shake or vortex the CleanNA Particles CCLV 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 Solution.
4. Add 270 µL CCLV Lysis, 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 CCLV.

8. Add 4 mL CCLV Binding. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.
9. Add 30 µL CleanNA Particles CCLV. 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 CCLV resuspended in solution.

11. Place the 24-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV. The Particles from each well will be collected by for magnets at the bottom.
12. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
14. Remove the 24-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
15. Add 1 mL CCLV Wash 1.
16. Resuspend the CleanNA Particles CCLV by vortexing for 5 minutes or pipetting up and down 20 times.

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

17. Transfer the resuspended CleanNA Particles CCLV 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 CCLV.
19. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
21. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
22. Add 1 mL CCLV Wash 1.
23. Resuspend the CleanNA Particles CCLV by vortexing for 5 minutes or pipetting up and down 20 times.

    Note: To obtain good purity, complete resuspension of the CleanNA Particles CCLV is critical.
24. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
25. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.

    Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
26. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
27. Add 1 mL CCLV Wash 2.

    Note: CCLV Wash 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.
28. Resuspend the CleanNA Particles CCLV 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 CCLV.
30. Incubate at room temperature until the CleanNA Particles CCLV are completely cleared from solution.
31. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CCLV.
32. Repeat Steps 26-31 for a second “CCLV Wash 2” wash step.
33. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
34. Place the 96-well Deep Well Plate on the 96 ring magnet plate to magnetize the CleanNA Particles CCLV.
35. Aspirate and discard the residual CCLV Wash 2.
36. Leave the tube on the magnetic separation device for 25 minutes to dry the CleanNA Particles CCLV.
37. Remove the 96-well plate containing the CleanNA Particles CCLV from the magnetic separation device.
38. Add 50-100 µL Elution Buffer. Resuspend the CleanNA Particles CCLV 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 CCLV.
41. Incubate at room temperature until the CleanNA Particles CCLV 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	Solution
Low DNA yield	Incomplete resuspension of CleanNA Particles CCLV.	Resuspend the CleanNA Particles CCLV by vortexing vigorously before use.
	Inefficient binding of the DNA to the CleanNA Particles CCLV.	Ensure to let the sample cool at room temperature for 10 minutes prior to addition of the CCLV Binding.
	Inefficient binding of the DNA to the CleanNA Particles CCLV.	Ensure to mix each sample continuously throughout the binding incubation.
	Loss of CleanNA Particles CCLV during operation.	Avoid disturbing the CleanNA Particles CCLV during aspiration.
	DNA remains bound to CleanNA Particles CCLV.	Increase elution volume and incubate at room temperature for 15 minutes; Pipet up and down 50 to 100 times.
	DNA washed off.	Dilute CCLV Wash 2 by adding appropriate volume of 100% ethanol prior to use (see Page 4 for instructions).
	Ethanol carryover.	Dry the CleanNA Particles CCLV at room temperature for 25 minutes before elution.
CleanNA particles CCLV do not completely clear from solution	Too short magnetizing time.	Increase collection time on the magnetic separation device.
High Molecular Weight Co- Purification	Two CCLV Wash 1 Steps must be performed.	Perform two CCLV Wash 1 steps as instructed in the manual. Increase the volume of wash buffer if necessary.
Problems in downstream applications	Salt carryover.	CCLV Wash 2 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 CCLV.
	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 CCLV.

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
Clean Circulating LV DNA Kit (384 Preps)	CCLV-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
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

Notes

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