Clean Blood LV DNA Kit

USER MANUAL

Manual revision v5.00

Catalog Numbers:
CBLV-D0024: 24 preps
CBLV-D0096: 96 preps

Batch No: See package

Shipping: room temperature

Storage and stability: CleanNA Particles LV and Proteinase K (20 mg/mL) 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 Blood 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 Blood 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

Contents

Introduction and Principle

The Clean Blood LV DNA Kit allows for the isolation of high-quality genomic DNA from up to 10 mL whole blood samples. The procedure allows for both manual as well as automated sample processing.

Our CleanNA Particles LV offer a high binding capacity and a fast-magnetic response time, thereby decreasing the overall processing time. Utilizing our CleanNA paramagnetic particles, we provide a fast and convenient method for genomic DNA isolation from fresh blood, frozen blood and buffy coats.

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 for downstream applications using an Elution Buffer.

The isolated DNA is ready for immediate use in many downstream applications, such as Next Generation Sequencing, PCR amplification and enzymatic reactions.

Kit Contents and Materials

Kit Contents:

Product	CBLV-D0024	CBLV-D0096	Storage
Preps	24	96	n/a
WBC Lysis	360 mL	2 x 725 mL	15-25°C
Proteinase K (20 mg/mL)	28 mL	90 mL	15-25°C (for storage > 12 months, store at 2-8 °C)
CHB Binding	100 mL	2 x 200 mL	15-25°C
CleanNA Particles LV	13 mL	40 mL	2-8°C
ULG Wash	220 mL	2 x 440 mL	15-25°C
Elution Buffer	250 mL	1000 mL	15-25°C

Materials and Reagents to be supplied by User:

- 50 mL centrifuge tube compatible with the magnetic separation device
- Magnetic separation device for 50 mL tubes
- 15 mL centrifuge tube compatible with the magnetic separation device
- Magnetic separation device for 15 mL tubes
- Vortexer
- Heat block, incubator, or water bath capable to be set at 70°C
- Microcentrifuge tubes for DNA storage
- Ethanol absolute
- 70% ethanol
- 100% isopropanol
- Molecular biology grade water
- Optional: RNase A (25 mg/mL)
- Optional: PBS

Preparation of Reagents

CHB Binding
Prepare CHB Binding with 100% Isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
CBLV-D0024	400 mL
CBLV-D0096	800 mL per bottle

ULG Wash
Prepare ULG Wash with Ethanol absolute as follows and store at room temperature.

Kit	Ethanol absolute to be Added
CBLV-D0024	280 mL
CBLV-D0096	560 mL per bottle

70% Ethanol
Prepare stock of 70% ethanol and store at room temperature.

Kit	Total Amount of 70% Ethanol Required	
	5 mL Blood Sample	**10 mL Blood Sample**
CBLV-D0024	110 mL	220 mL
CBLV-D0096	400 mL	800 mL

Shake or vortex the CleanNA Particles LV to fully resuspend the particles prior to use. The particles must be fully suspended during use to ensure proper binding.

Protocol for 2 mL Blood

Before Starting:

- Set heat block, incubator, or water bath to 70°C.
- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Heat Elution Buffer to 70°C

Protocol:

1. Add a 2 mL blood sample to a 15 mL centrifuge tube (not provided). Bring the volume up to 2 mL with PBS (not provided) if volume of blood is less than 2 mL.
2. Prepare a mastermix of WBC Lysis and Proteinase K (20 mg/mL) only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
WBC Lysis	2.32 mL	61.3 mL
Proteinase K (20 mg/mL)	160 µL	4.2 mL

3. Add 2.48 mL WBC Lysis/Proteinase K (20 mg/mL) mastermix to each sample. Vortex for 1 minute or pipet up and down 20 times to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols, tip mixing yields best results and is recommended.

4. Incubate at 70°C for 30 minutes.
5. Incubate at room temperature for 10 minutes to cool the sample.

Optional: To ensure RNA removal, 40 µL RNase A (25 mg/mL) can be added to each sample (not provided). Vortex or pipet up and down 20 times to mix. For automated protocols, tip mixing yields best results and is recommended.

6. Add 3.2 mL CHB Binding and 80 µL CleanNA Particles LV to each sample. Vortex for 20 minutes to mix.

   Note: CHB Binding must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. CHB Binding and CleanNA Particles LV can be prepared as a mastermix. Mix only what is needed for immediate processing.

   Note: If constant vortexing for 20 minutes is not possible, vortex for 30 seconds every 2-3 minutes for 20 minutes.

7. Place the tube on a magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature for 10 minutes until the CleanNA Particles LV are completely cleared from solution.

   Note: Time may be increased or decreased depending on the strength of the magnet used.

8. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
9. Remove the tube from the magnetic separation device.
10. Add 800 µL ULG Wash to each sample.

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

11. Vortex for 1 minute.

    Note: Complete resuspension of the CleanNA Particles LV is critical for obtaining good purity.

12. 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.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second ULG Wash step.
16. Add 800 µL 70% ethanol (not provided) to each sample.
17. Vortex for 1 minute or pipet up and down 20 times to mix.
18. 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.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
20. Repeat Steps 16-19 for a second 70% ethanol wash step.
21. Leave the tube on the magnetic separation device. Add 400 µL molecular biology grade water (not provided) and immediately aspirate. Do not leave the molecular biology grade water on CleanNA Particles LV for more than 60 seconds.
22. Remove the tube from the magnetic separation device.
23. Add 200-400 µL Elution Buffer preheated to 70°C to elute DNA from the CleanNA Particles LV.

    Note: Heat Elution Buffer to 70°C to improve yield.
24. Pipet to mix and incubate at for 5 minutes.
25. 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.
26. Transfer the cleared supernatant containing purified DNA to a microcentrifuge tube (not provided). Store DNA at -20°C.

Protocol for 5 mL Blood

Before Starting:

- Set heat block, incubator, or water bath to 70°C.
- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Heat Elution Buffer to 70°C

Protocol:

1. Add a 5 mL blood sample to a 50 mL centrifuge tube (not provided). Bring the volume up to 5 mL with PBS (not provided) if volume of blood is less than 5 mL.
2. Prepare a mastermix of WBC Lysis and Proteinase K (20 mg/mL) only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
WBC Lysis	5.8 mL	153.1 mL
Proteinase K (20 mg/mL)	400 µL	10.5 mL

3. Add 6.2 mL WBC Lysis/Proteinase K (20 mg/mL) mastermix to each sample. Vortex for 1 minute or pipet up and down 20 times to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols, tip mixing yields best results and is recommended.

4. Incubate at 70°C for 30 minutes.
5. Incubate at room temperature for 10 minutes to cool the sample.

Optional: To ensure RNA removal, 100 µL RNase A (25 mg/mL) can be added to each sample (not provided). Vortex or pipet up and down 20 times to mix. For automated protocols, tip mixing yields best results and is recommended.

6. Add 8 mL CHB Binding and 200 µL CleanNA Particles LV to each sample. Vortex for 20 minutes to mix.

CHB

Note: CHB Binding must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. Binding and CleanNA Particles LV can be prepared as a mastermix. Mix only what is needed for immediate processing.

Note: If constant vortexing for 20 minutes is not possible, vortex for 30 seconds every 2-3 minutes for 20 minutes.

7. Place the tube on a magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature for 10 minutes until the CleanNA Particles LV are completely cleared from solution.

   Note: Time may be increased or decreased depending on the strength of the magnet used.

8. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
9. Remove the tube from the magnetic separation device.
10. Add 2 mL ULG Wash to each sample.

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

11. Vortex for 1 minute.

    Note: Complete resuspension of the CleanNA Particles LV is critical for obtaining good purity.

12. 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.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second ULG Wash step.
16. Add 2 mL 70% ethanol (not provided) to each sample.
17. Vortex for 1 minute or pipet up and down 20 times to mix.
18. 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.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
20. Repeat Steps 16-19 for a second 70% ethanol wash step.
21. Leave the tube on the magnetic separation device. Add 1 mL molecular biology grade (not provided) and immediately aspirate. Do not leave the molecular biology grade on CleanNA Particles LV for more than 60 seconds.
22. Remove the tube from the magnetic separation device.
23. Add 0.5-1 mL Elution Buffer preheated to 70°C to elute DNA from the CleanNA Particles LV.

    Note: Heat Elution Buffer to 70°C to improve yield.
24. Pipet to mix and incubate at for 5 minutes.
25. 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.
26. Transfer the cleared supernatant containing purified DNA to a microcentrifuge tube (not provided). Store DNA at -20°C.

Protocol for 10 mL Blood

Before Starting:

- Set heat block, incubator, or water bath to 70°C.
- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Heat Elution Buffer to 70°C

Protocol:

1. Add a 10 mL blood sample to a 50 mL centrifuge tube (not provided). Bring the volume up to 10 mL with PBS (not provided) if volume of blood is less than 10 mL.
2. Prepare a mastermix of WBC Lysis and Proteinase K (20 mg/mL) only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
WBC Lysis	11.6 mL	306 mL
Proteinase K (20 mg/mL)	800 µL	21 mL

3. Add 12.4 mL WBC Lysis/Proteinase K (20 mg/mL) mastermix to each sample. Vortex for 1 minute or pipet up and down 20 times to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols, tip mixing yields best results and is recommended.
4. Incubate at 70°C for 30 minutes.
5. Incubate at room temperature for 10 minutes to cool the sample.

   Optional: To ensure RNA removal, 200 µL RNase A (25 mg/mL) can be added to each sample (not provided). Vortex or pipet up and down 20 times to mix. For automated protocols, tip mixing yields best results and is recommended.
6. Add 16 mL CHB Binding and 400 µL CleanNA Particles LV to each sample. Vortex for 20 minutes to mix.

   Note: CHB Binding must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. CHB Binding and CleanNA Particles LV can be prepared as a mastermix. Mix only what is needed for immediate processing.

   Note: If constant vortexing for 20 minutes is not possible, vortex for 30 seconds every 2-3 minutes for 20 minutes.
7. Place the tube on a magnetic separation device to magnetize the CleanNA Particles LV. Incubate at room temperature for 10 minutes until the CleanNA Particles LV are completely cleared from solution.

   Note: Time may be increased or decreased depending on the strength of the magnet used.
8. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
9. Remove the tube from the magnetic separation device.
10. Add 4 mL ULG Wash to each sample.

    Note: ULG Wash must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.
11. Vortex for 1 minute.

    Note: Complete resuspension of the CleanNA Particles LV is critical for obtaining good purity.
12. 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.

13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second ULG Wash wash step.
16. Add 4 mL 70% ethanol (not provided) to each sample.
17. Vortex for 1 minute or pipet up and down 20 times to mix.
18. 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.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles LV.
20. Repeat Steps 16-19 for a second 70% ethanol wash step.
21. Leave the tube on the magnetic separation device. Add 1 mL molecular biology grade (not provided) and immediately aspirate. Do not leave the molecular biology grade on CleanNA Particles LV for more than 60 seconds.
22. Remove the tube from the magnetic separation device.
23. Add 1-2 mL Elution Buffer preheated to 70°C to elute DNA from the CleanNA Particles LV.

 **Note:** Heat Elution Buffer to 70°C to improve yield.

24. Pipet to mix and incubate at for 5 minutes.
25. 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.
26. Transfer the cleared supernatant containing purified DNA to a microcentrifuge tube (not provided). 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 Solutions

Problem	Cause	Solution
Low DNA yield	Poor resuspension of CleanNA Particles LV.	Resuspend the CleanNA Particles LV by vortexing vigorously before use.
	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 65°C for 15 minutes; pipette up and down 50 to 100 times. Pre-heat the elution buffer to 70°C.
	Ethanol is not added into ULG Wash.	Make sure to add ethanol to the ULG Wash (see Page 4 for instructions).
	Improperly mixed frozen blood samples after thawing.	Thaw the frozen blood at room temperature and gently mix the blood by inverting.
	Inefficient cell lysis due to inefficient mixing of Lysis Buffer and Sample.	Ensure the sample is thoroughly mixed with Lysis Buffer and Proteinase K (20 mg/mL). Extend incubation by 10 minutes.
CleanNA Particles LV do not completely clear from solution	Separation time on the magnet device was too short.	Increase collection time on the magnet.
Gel-like material in the eluted DNA	Blood is too old.	Use 8 mM NaOH as elution buffer.
		Remove the gel-like material by centrifugation; recommend using fresh blood.
Problems in downstream applications	Salt carryover.	ULG Wash and 70% ethanol must be at room temperature.
	Ethanol carryover.	Dry the CleanNA Particles LV CleanNA Particles before elution.

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Blood LV DNA Kit (24 Preps)	CBLV-D0024
Clean Blood LV DNA Kit (96 Preps)	CBLV-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
5.00	03/DEC/2024	Total revision	Updated buffer names and Proteinase K volume from 25 to 28 mL.
4.00	October 2021	Total revision	Revised layout.
			Overall clearer language.
3.00	August 2020	Total revision	New layout.
		User manual information.	General heading before contents added.