Clean Blood LV DNA Kit

USER MANUAL

Manual revision v4.00

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

Batch No: See package

Shipping: Room temperature

Storage and stability: CleanNA Particles BLV and Proteinase K Solution 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 CleanNA 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 BLV 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
BLV Lysis Buffer	360 mL	2 x 725 mL	15-25°C
Proteinase K Solution	25 mL	90 mL	2-8°C
BLV Binding Buffer	100 mL	2 x 200 mL	15-25°C
CleanNA Particles BLV	10 mL	40 mL	2-8°C
BLV Wash Buffer	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
- 100% ethanol
- 70% ethanol
- 100% isopropanol
- Molecular biology grade water
- Optional: RNase A (25 mg/mL)
- Optional: PBS

Preparation of Reagents

BLV Binding Buffer
Prepare BLV Binding Buffer 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

BLV Wash Buffer
Prepare BLV Wash Buffer with 100% Ethanol as follows and store at room temperature.

Kit	100% Ethanol 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 BLV 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 BLV Lysis Buffer and Proteinase K Solution only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
BLV Lysis Buffer	2.32 mL	61.3 mL
Proteinase K Solution	160 µL	4.2 mL

3. Add 2.48 mL BLV Lysis Buffer/Proteinase K Solution 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 BLV Binding Buffer and 80 µL CleanNA Particles BLV to each sample. Vortex for 20 minutes to mix.

Note: BLV Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. BLV Binding Buffer and CleanNA Particles BLV 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 BLV. Incubate at room temperature for 10 minutes until the CleanNA Particles BLV 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 BLV.
9. Remove the tube from the magnetic separation device.
10. Add 800 µL BLV Wash Buffer to each sample.

Note: BLV Wash Buffer 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 BLV is critical for obtaining good purity.

12. Place the tube on the magnetic separation device to magnetize the CleanNA Particles BLV. Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second BLV Wash Buffer 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 BLV. Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
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 BLV 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 BLV.

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 BLV. Incubate at room temperature until the CleanNA Particles BLV 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 BLV Lysis Buffer and Proteinase K Solution only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
BLV Lysis Buffer	5.8 mL	153.1 mL
Proteinase K Solution	400 µL	10.5 mL

3. Add 6.2 mL BLV Lysis Buffer/Proteinase K Solution 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 BLV Binding Buffer and 200 µL CleanNA Particles BLV to each sample. Vortex for 20 minutes to mix.

Note: BLV Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. BLV Binding Buffer and CleanNA Particles BLV 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 BLV. Incubate at room temperature for 10 minutes until the CleanNA Particles BLV 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 BLV.
9. Remove the tube from the magnetic separation device.
10. Add 2 mL BLV Wash Buffer to each sample.

Note: BLV Wash Buffer 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 BLV is critical for obtaining good purity.

12. Place the tube on the magnetic separation device to magnetize the CleanNA Particles BLV. Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.
13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second BLV Wash Buffer 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 BLV. Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
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 BLV 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 BLV.

     **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 BLV. Incubate at room temperature until the CleanNA Particles BLV 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 BLV Lysis Buffer and Proteinase K Solution only for the samples to be extracted according to the table below:

Component	Amount Per Prep	Total Amount Per 24-well plate
BLV Lysis Buffer	11.6 mL	306 mL
Proteinase K Solution	800 µL	21 mL

3. Add 12.4 mL BLV Lysis Buffer/Proteinase K Solution 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 BLV Binding Buffer and 400 µL CleanNA Particles BLV to each sample. Vortex for 20 minutes to mix.

   

   Note: BLV Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. BLV Binding Buffer and CleanNA Particles BLV 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 BLV. Incubate at room temperature for 10 minutes until the CleanNA Particles BLV 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 BLV.
9. Remove the tube from the magnetic separation device.
10. Add 4 mL BLV Wash Buffer to each sample.

    

    Note: BLV Wash Buffer 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 BLV is critical for obtaining good purity.
12. Place the tube on the magnetic separation device to magnetize the CleanNA Particles BLV.

Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.

13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
14. Remove the tube from the magnetic separation device.
15. Repeat Steps 10-14 for a second BLV Wash Buffer 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 BLV. Incubate at room temperature until the CleanNA Particles BLV are completely cleared from solution.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles BLV.
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 BLV 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 BLV.

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 BLV. Incubate at room temperature until the CleanNA Particles BLV 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 BLV.	Resuspend the CleanNA Particles BLV by vortexing vigorously before use.
	Loss of CleanNA Particles BLV during operation.	Avoid disturbing the CleanNA Particles BLV during aspiration.
	DNA remains bound to CleanNA Particles BLV.	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 BLV Wash Buffer.	Make sure to add ethanol to the BLV Wash Buffer (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 solution. Extend incubation by 10 minutes.
CleanNA particles BLV 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.	BLV Wash Buffer and 70% ethanol must be at room temperature.
	Ethanol carryover.	Dry the CleanNA Particles BLV 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-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
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.

Notes

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Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com