Clean Plasma cfDNA Kit Dx

Instructions for Use

V.1 - AUGUST 2026

CE IVD

REF CPCF2Dx-D0096

CleanNA, Coenecoop 75, 2741 PH, Waddinxveen, The Netherlands

Intended for in vitro diagnostic use.

Information in this document is subject to change without notice. Regularly visit www.cleanna.com/download-cpcfdx to check for updates in this document.

Disclaimer

CleanNA disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. To the fullest extend allowed by law, in no event shall CleanNA be liable, whether contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequental damages in connection with or arising from this document, including, but not limited to the use thereof, whether or not forseeable and whether or not CleanNA is advised of the possibility of such damages.

Trademarks

The trademarks mentioned herein are the property of their respective owners.

Contents

Intended Purpose

The intended purpose of the Clean Plasma cfDNA Kit is to extract cell-free DNA from human plasma in a sufficient purity to be used in downstream detection procedures based on the principle of Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS). In addition, the device is able to extract cell-free DNA from cerebrospinal fluid. It can be used manually or on automated extraction devices that can handle magnetic particles.

Intended User

The Clean Plasma cfDNA Kit Dx is intended to be used by professionals who are trained in molecular biology laboratory techniques.

Introduction and Principle

The Clean Plasma cfDNA Kit Dx allows for the extraction of cell-free DNA from sample volumes up to 2 mL plasma or cerebrospinal fluid. The kit combines our proprietary buffer system with the convenience of the magnetic Cell-Free Particles. As a result, the Clean Plasma cfDNA Kit Dx provides a procedure that can be used in automated protocols via a simple 4-step process (lyse, bind, wash and elute).

Our Cell-Free Particles 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 Cell-Free Particles decreases the amount of particles required during binding steps, thereby reducing the elution volume. This enables isolated cell free DNA from 2 mL sample to be eluted in as little as 40 µL.

The protocol is scalable due to the use of our magnetic bead purification technology and can, besides manual usage, easily be automated on our CleanXtract series or liquid handling workstations. Extracted 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 cell-free DNA from proteins and vesicles bound to the DNA while DNases are inactivated. DNA is isolated from the lysate in one step by binding to magnetic particles' surface. The Cell-Free 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 Cell-Free Particles using Elution Buffer LT.

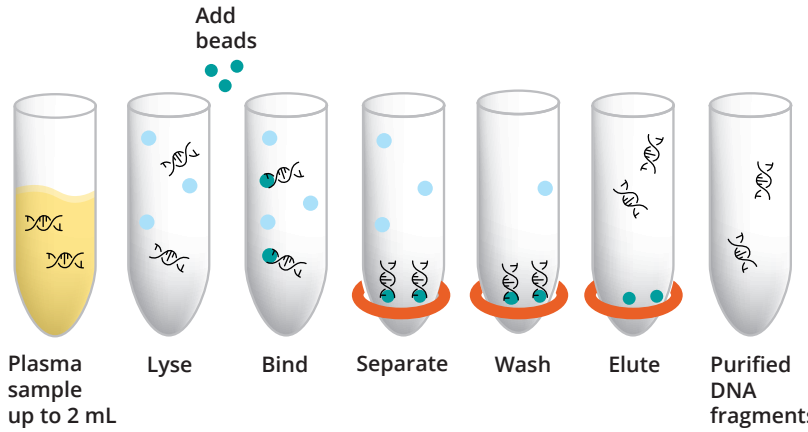


Figure 1: Schematic overview of the Clean Plasma cfDNA Kit Dx extraction procedure.

Materials Provided

Kit Contents:

Component	CPCF2Dx-D0096 (96 preps)
Cell-Free Particles	2,1 mL
CFL Buffer	15 mL
DB Buffer	225 mL
IG Wash	162 mL
Elution Buffer LT	100 mL
Proteinase K (20 mg/mL)	3,2 mL

Number of reactions per protocol

Protocol	Number of reactions from 96 prep kit CPCF2Dx-D0096
Manual Protocol - 1 mL Input Volume	126
0,5 mL Input Volume - CleanXtract 96	384
1 mL Input Volume - CleanXtract LV	192
2 mL Input Volume - CleanXtract LV	96

Reagent Shipping, Storage and Handling

The Clean Plasma cfDNA Kit Dx is shipped at room temperature (15-25 °C). Do not freeze the components of the Clean Plasma cfDNA Kit Dx. After the components have been frozen, the kit is no longer suitable for use.

The in-use stability for the kit is one week (8 days) after opening. Do not use the Clean Plasma cfDNA Kit Dx after the expiration date stated on the outer box label.

Component	Storage Temperature
Cell-Free Particles	2-8 °C
CFL Buffer	15-25 °C
DB Buffer	15-25 °C
IG Wash	15-25 °C
Elution Buffer LT	15-25 °C
Proteinase K (20 mg/mL)	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.

Any serious incident that has occured in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Explanation of LOT number use on the product labels:

- The Cell-Free Particles and Proteinase K **boxes** contain the **LOT number of the kit** the component is part of (for traceability).
- The Cell-Free Particles and Proteinase K **bottles** contain the **LOT number of the component itself**.

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).

CFL 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.
DB Buffer	
	Flammable liquid and vapour. Causes serious eye damage. Harmful if swallowed. Causes skin irritation. Harmful to aquatic life with long lasting effects. Contact with acids liberates vey toxic gas. **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** (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.
IG 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]. Wash contaminated clothing before reuse. **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. 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 Plasma cfDNA Kit Dx 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 and EN-ISO 9001certified.

Limitations

The performance of the Clean Plasma cfDNA Kit Dx has been established in studies where DNA was extracted from human plasma that has been frozen and preserved with one of the following anti-coagulants:

- EDTA
- Citrate-phosphate-dextrose (CPD)

A range of individual plasma donors and cerebrospinal fluid donors has been included in the performance evaluation. The performance of the Clean Plasma cfDNA Kit Dx has not been tested with heamolysed plasma.

It is the user's responsibility to validate the performance of sample material not used in the performance evaluation.

We recommend the use of an internal extraction control per sample to identify a false negative result in downstream detection methods, caused by potentially unknown inhibitory agents in samples.

The performance of the kit has been established with downstream detection methods based on PCR and NGS. It is the user's responsibility to validate the performance of the device when used with other downstream detection methods.

Diagnostic results generated after using the Clean Plasma cfDNA Kit Dx must be interpreted in conjunction with other clinical or laboratory findings.

Collection, Storage and Handling of Specimen

The procedures below describe the sample handling during the performance studies of the Clean Plasma cfDNA Kit Dx.

The collected plasma is stored with either EDTA or CPD as anticoagulant and frozen at -80 ºC until further use. Cerebrospinal fluid is frozen without anticoagulants.

Sample preparation:

1. Take the sample out of the freezer.
2. Set a centrifuge at 4°C.
3. Once the sample is thawed, invert the tube(s) at least ten times.
4. Place the sample tube(s) into the centrifuge.

⚠ **Note:** Make sure the centrifuge is balanced correctly.

5. Spin the sample tube(s) for 10 minutes at 4.000 rpm (2.000 rpm for cerebrospinal fluid) at 4°C.
6. Carefully take out the sample tube(s).
7. Transfer the entire volume clean supernatant to a new tube.
8. Invert the newly transferred supernatant at least ten times.

Materials and Equipment to be Supplied by User

For manual protocols

- Absolute Ethanol
- Absolute Isopropanol
- Vortexer
- Centrifuge
- Thermoshaker capable to be set at 60°C
- Magnetic seperation device for 1,5/2,0 mL tubes
- 2,0 mL microcentrifuge tube(s)

For automated (CleanXtract LV) protocols

- Absolute Ethanol
- Absolute Isopropanol
- Vortexer
- Centrifuge
- Thermoshaker capable to be set at 60°C
- CleanXtract LV cartridges, bores and elution tubes
- Plastic (cartridge) seals
- CleanXtract LV Instructions for Use
- 2,0 mL microcentrifuge tube(s)

For automated (CleanXtract 96) protocols

- Absolute Ethanol
- Absolute Isopropanol
- Vortexer
- Centrifuge
- Thermoshaker capable to be set at 60°C
- CleanXtract 96 plates and comb
- Plastic (plate) seals
- CleanXtract 96 Instructions for Use
- 5,0 mL tube(s)

Preparation of Reagents

IG Wash

Prepare IG Wash with Isopropanol as follows and store at room temperature.

Kit	Isopropanol to be added
CPCF2Dx-D0096	69 mL

Manual Protocol

1 mL Input Volume

Before Starting:

• Prepare samples according to 'Collection, Storage and Handling of Specimen' on page 10.
• Make sure the Cell-Free Particles and Proteinase K (20 mg/mL) are at room temperature.
• Set thermoshaker at 60°C.
• Prepare IG Wash according to the instructions in the Preparation of Reagents section on Page 12.
• Prepare a fresh 80% ethanol solution.
• Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting.

Protocol:

1. Prepare a CPCF Lysis mix in a tube according to Table 1.

Table 1: CPCF Lysis mix preparation.

Reagent	1 Sample	10 Samples*
CFL Buffer	67 µL	704 µL
Proteinase K	15 µL	158 µL
Total Volume	82 µL	862 µL

* Includes 5% excess volume

2. Pipet up and down until the solution looks clear. Avoid creating bubbles by pipetting too quickly.
3. Add up to 1 mL sample to a 2,0 mL microcentrifuge tube.

⚠ **Note:** Do not exceed the maximum sample volume, this will decrease the efficiency of the extraction procedure.

4. Bring the sample volume up to 1 mL with Elution Buffer LT (provided with this kit) if the sample volume is less than 1 mL.
5. Add 82 µL of the CPCF Lysis mix to each sample.
6. Put the samples in a thermoshaker (1000 rpm) at 60°C for 20 minutes.
7. After taking out the samples, change the settings of the thermoshaker to 20 °C for a later step.
8. Incubate the samples 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 Cell-Free Particles.

9. Add 1000 µL DB Buffer.
10. Add 15 µL Cell-Free Particles. Invert the sample 10 times or pipet up and down to mix.

⚠ **Note:** Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting **each sample.**

11. Put the samples in the thermoshaker (1500 rpm) at 20°C for 10 minutes.
12. Place the tube on a magnetic separation device to magnetize the Cell-Free Particles. Incubate until the Cell-Free Particles are completely cleared from solution.

⚠ **Note:** Make sure to incubate until all particles are cleared from solution, bead loss can cause lower yield.

13. Aspirate and discard the cleared supernatant.

⚠ **Note:** Do not disturb or pipet the Cell-Free Particles. This can cause lower yield.

14. Remove the tubes from the magnetic separation device.
15. Add 500 µL IG Wash to each tube.

⚠ **Note:** IG Wash must be diluted with Isopropanol prior to use. Please see Page 12 for instructions.

16. Put the samples in a thermoshaker (1500 rpm) at 20°C for 2 minutes.

⚠ **Note:** To obtain good purity, complete resuspension of the Cell-Free Particles is critical.

17. Place the tube on the magnetic separation device to magnetize the Cell-Free Particles. Incubate at room temperature until the Cell-Free Particles are completely cleared from solution.
18. Aspirate and discard the cleared supernatant. Do not disturb the Cell-Free Particles.
19. Remove the tubes containing the Cell-Free Particles from the magnetic separation device.
20. Repeat steps 15-19 for a second IG Wash step.
21. Add 500 µL 80% ethanol to the tubes.
22. Put the samples in a thermoshaker (1500 rpm) at 20°C for 2 minutes.
23. Place the tube on the magnetic separation device to magnetize the Cell-Free Particles. Incubate until the Cell-Free Particles are completely cleared from solution.
24. Aspirate and discard the cleared supernatant. Do not disturb the Cell-Free Particles.
25. Let the particles air-dy for 5 minutes on the magnet. After the first minute, aspirate any residual 80% ethanol.

⚠ **Note:** Do not overdry the particles. This can cause lower yield.

26. Remove the tube containing the Cell-Free Particles from the magnetic separation device.
27. Add 60 µL Elution Buffer LT. Resuspend the Cell-Free Particles by pipetting up and down 10 times.
28. Put the samples in a thermoshaker (1500 rpm) at 20°C for 10 minutes.

29. Place the tube on the magnetic separation device to magnetize the Cell-Free Particles. Incubate at room temperature until the Cell-Free Particles are completely cleared from solution.
30. Transfer the cleared supernatant containing purified cfDNA to a clean PCR tube (not provided).
31. Store the DNA at -20°C.

Automated Protocols

0,5 mL Input Volume - CleanXtract 96

Before Starting:

• Prepare samples according to 'Collection, Storage and Handling of Specimen' on page 10.
• Make sure the Cell-Free Particles and Proteinase K (20 mg/mL) are at room temperature.
• Turn on the CleanXtract 96 and use the UV function for 30 minutes.
• Prepare IG Wash according to the instructions in the Preparation of Reagents section on Page 12.
• Prepare a fresh 80% ethanol solution.
• Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting.

Lysis CleanXtract 96 run:

1. Take two 2,2 mL 96-well Deep-well plates.
2. Take one of the 2,2 mL 96-well Deep-well plates, label it "Tip-comb" and place a 96-well Tip-comb into this plate.
3. Take the other 2,2 mL plate and label it "Lysis/Binding".
4. Add 500 µL sample to each well. Bring the sample volume up to 500 µL with Elution Buffer LT if the sample volume is less than this.

⚠ **Note:** Do not exceed the maximum sample volume, this will decrease the efficiency of the extraction procedure.

5. Prepare a CPCF Lysis mix in a tube according to Table 2.

Table 2: CPCF Lysis mix preparation.

Reagent	1 Sample	96 Samples*
CFL Buffer	33,5 µL	3377 µL
Proteinase K	7,5 µL	756 µL
Total Volume	41 µL	4133 µL

*Includes 5% excess volume

6. Invert the tube containing the CPCF Lysis mix by inverting at least 10 times.
7. Add 41 µL of the CPCF Lysis mix to each sample.
8. Select "Run Setting" on the CleanXtract 96 in the middle of the display of the CleanXtract 96.
9. After pressing "Run Setting", the various positions on the CleanXtract 96 are shown.

10. Select “Position 2”, the CleanXtract 96 will turn the position to the right side of the device. Open the blue cover to load the “Tip-comb” plate into the device as mentioned in Figure 2.

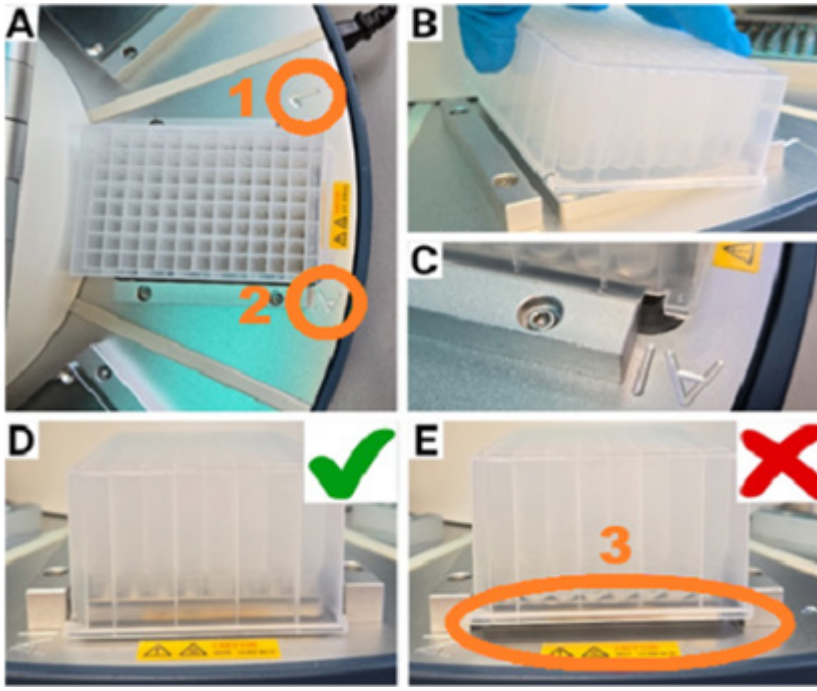


Figure 2: (A) 2,2 mL 96-well Deep-well plate placed on the right side of the CleanXtract 96. The plate position of the CleanXtract 96 is indicated with circle 1 and the A1 position is highlighted with circle 2. (B) How to place the 2,2 mL 96-well Deep-well plate into the CleanXtract 96. (C) Notch of the 2,2 mL 96-well Deep-well plate should be in the correct position. (D) Correct placement of the 2,2 mL 96-well Deep-well plate. (E) Incorrect placement of the 2,2 mL 96-well Deep-well plate, with the space between the position and the plate highlighted with circle 3.

11. Repeat this process for all the plates as indicated in Table 3 and Figure 3.

Table 3: Plate positions for the CPCF lysis protocol on the CleanXtract 96.

Position on CleanXtract 96	Plate ID	Containing
2	(1) Tip-comb	96-well Tip-comb
1	(2) Lysis/Binding	41 µL CPCF Lysis mix + 500 µL Sample

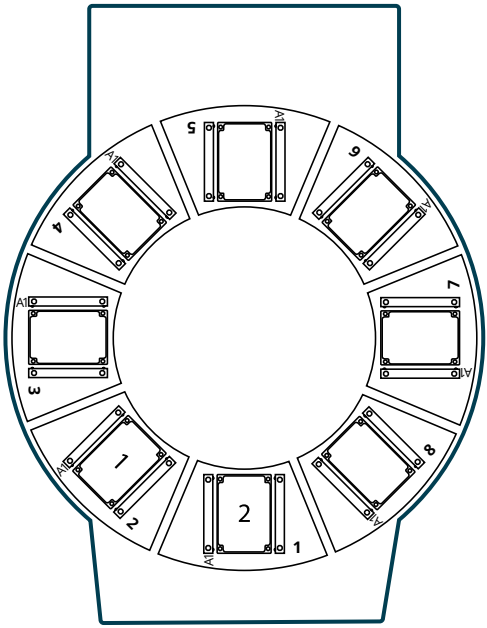


Figure 3: View from above on the plate positions in the CleanXtract 96.

12. Close the blue cover on the right side of the CleanXtract 96 and press the "<-" on the top left corner of the display.
13. Press "File Management" on the main interface of the CleanXtract 96.
14. Select the "CPCF" folder and then select the "CXT96_CPCF_PL500_LS" protocol.

⚠ **Note:** The folder and protocol will be highlighted in light blue after they are selected.

15. Press "Open" in the bottom right corner of the display.
16. In the protocol screen, select "Prepare for Running".
17. The CleanXtract 96 will ask if the tip-comb is installed before starting. Double check if the tip-comb is installed at **position 2** before selecting "Yes".
18. The protocol will now start.
19. While the protocol is running, take four 2,2 mL 96-well Deep-well plates and label and fill them according to the plate ID and volume in Table 4.

Table 4: Required 2,2 mL 96-well Deep-well plates for the CPCF extraction

Plate ID	Reagent used	Volume
Wash 1	IG Wash	250 µL
Wash 2	80% ethanol	250 µL
Wash 3	80% ethanol	250 µL
Elution	Elution Buffer LT	50 µL

20. After the protocol is finished, take out the “Lysis/Binding” plate and seal it with a general seal.
21. Now take out the “Tip-comb” plate and keep this plate for the extraction.
22. Let the “Lysis/Binding” plate cool down for at least 10 minutes before continuing to the extraction.

Extraction CleanXtract 96 run:

1. During the “Lysis/Binding” plate cooldown prepare a CPCF Binding mix in a tube for all your reactions according to Table 5 in one tube.

Table 5: Method to create a CPCF Binding mix.

Reagent	1 reaction	96 Samples*
DB Buffer	500 µL	50,4 mL
Cell-Free Particles	5 µL	504 µL
Total Volume	505 µL	50,9 mL

* Includes 5% excess volume

⚠ **Note:** Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting each sample.

2. Invert the tube(s) with the CPCF Binding mix at least ten times.
3. When the “Lysis/Binding” plate has been incubating at RT for at least 10 minutes, the CPCF Binding mix can be added. Remove seal and transfer 505 µL of CPCF Binding mix to each sample.

⚠ **Note:** Invert the CPCF Binding mix at least ten times prior to use and after 4 samples.

4. Select “Run Setting” on the CleanXtract 96 in the middle of the main interface.
5. After pressing “Run Setting”, the various positions on the CleanXtract 96 are shown.
6. Select “Position 1”, the CleanXtract 96 will turn the position to the right side of the device. Open the blue cover to load the “Tip-comb” plate into the device.
7. Repeat this process for all the plates as indicated in Table 6 and Figure 4.

Table 6: Plate positions for the CPCF extraction protocol on the CleanXtract 96.

Position on the CleanXtract 96	Plate ID	Containing
1	(1) Tip-comb	96-well Tip-comb
2	(2) Lysis/Binding	541 µL lysate + 505 CPCF Binding mix
3	(3) Wash 1	250 µL IG Wash
4	(4) Wash 2	250 µL 80% ethanol
5	(5) Wash 3	250 µL 80% ethanol
6	(4) Elution	50 µL Elution Buffer LT

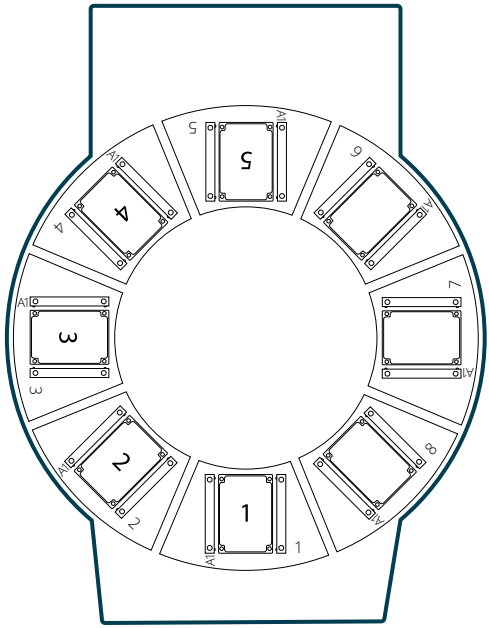


Figure 4: View from above on the plate positions in the CleanXtract 96.

⚠ **Note:** The Tip-comb plate from the lysis during the sample preparation can be reused.

8. Close the blue cover on the right side of the CleanXtract 96 and press the "<-" on the top left corner of the display.
9. Press "File Management" on the main interface of the CleanXtract 96.
10. Select the "CPCF" folder and then select the "CXT96_CPCF_PL500_EX" protocol.
11. Press "Open" in the bottom right corner of the display.
12. The protocol will now open, and it will show all the steps as shown in Figure 4.
13. Select "Prepare for Running".
14. The CleanXtract 96 will ask if the tip-comb is installed before starting. Double check if the tip-comb is installed at **position 1** before selecting "Yes".
15. The protocol will now start.
16. After the protocol is finished, unload the plates.
17. Seal the elution plate or tranfer the eluates to new storage tubes. Store the DNA at -20 °C.

1 mL Input Volume - CleanXtract LV

Before Starting:

• Prepare samples according to 'Collection, Storage and Handling of Specimen' on page 10.
• Turn on the CleanXtract LV and use the UV function for 20 minutes.
• Set the thermoshaker at 60°C.
• Prepare IG Wash according to the instructions in the Preparation of Reagents section on Page 12.
•Prepare a fresh 80% ethanol solution.
• Make sure the Cell-Free Particles and Proteinase K (20 mg/mL) are at room temperature.
• Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting.

Sample Preparation:

1. Add 1 mL sample to a 2,0 mL microcentrifuge tube (if the sample volume is less, fill up to 1 mL with Elution Buffer LT).

⚠ **Note:** Do not exceed the maximum sample volume, this will decrease the efficiency of the extraction procedure.

2. Prepare a CPCF lysis mix in a separate tube according to Table 7 and mix by inverting the tube for at least ten times.

Table 7: CPCF Lysis mix preparation for 1 mL sample input.

Reagent	1 Sample	16 Samples*
CFL Buffer	67 µL	1126 µL
Proteinase K	15 µL	252 µL
Total	82 µL	1414 µL

*Includes 5% excess volume.

3. Add 82 µL of CPCF Lysis mix to each sample.
4. Place the tubes in the pre-heated shaker (60°C) for 20 minutes at 1000 rpm.
5. Take the tubes from the shaker and let them cool down for 10 minutes at room temperature.

6. Prepare the CPCF Binding mix in a separate tube according to Table 8.

Table 8: CPCF Binding mix preparation for 1 mL sample input.

Reagent	1 Sample	16 Samples*
DB Buffer	1000 µL	16,8 mL
CleanNA Particles LV	10 µL	168 µL
Total volume	1010 µL	17 mL

*Includes 5% excess volume

⚠ **Note:** Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

7. Position CleanNA CXT LV Cartridge(s) (Figure 6A) in the Cartridge Rack according to the number of sample(s) required, as shown in Figure 5D.

⚠ **Note:** The well with the protrusion is well 8, indicated in the orange circle.

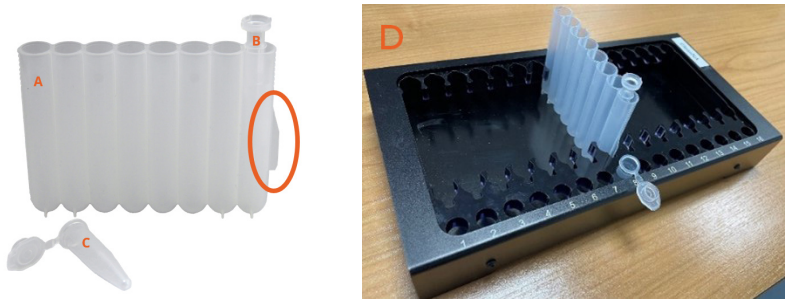


Figure 5: (A) = CleanNA CXT-LV Cartridge (B) = CleanNA CXT-LV Bore, (C) = CleanNA CXT-LV 1,5 mL Elution tube and (D) = Cartridge rack with one set of plastics.

8. Fill the cartridge(s) as mentioned in Table 9 and Figure 6.

Table 9: Plate positions for the CPCF extraction protocol on the CleanXtract LV.

Well number	Reagent used	Volume
1	CPCF Binding mix	1010 µL
2	IG Wash	500 µL
3	80% ethanol	500 µL
4	80% ethanol	500 µL
5	N/A	N/A
6	N/A	N/A
7	N/A	N/A
8	N/A	N/A

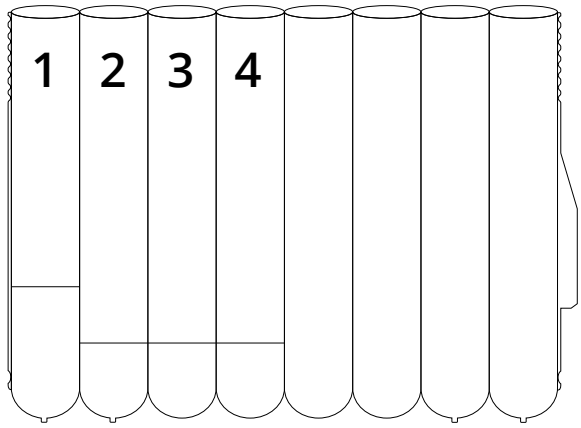


Figure 6: Side view on the filled CleanXtract LV cartidge.

⚠ **Note:** Shake or vortex the CPCF Binding mix to fully resuspend the particles before use. Invert the CPCF Binding mix at least ten times prior to use and after 4 samples.

⚠ **Note:** IG Wash must be diluted with Isopropanol prior to use. Please see Page 12 for instructions.

9. After preparing the cartridges, seal them with a plastic seal until the sample needs to be added.
10. Fill the CleanNA CXT-LV 1,5 mL Elution tube(s) (Figure 6C) with 30- 60 µL Elution Buffer LT.
11. Transfer the lysate (Sample + CPCF Lysis mix) to well 1 of the CleanNA CXT-LV Cartridge (this well already contains the CPCF Binding Mix).
12. Place a CleanNA CXT-LV Bore in well 8 (Figure 6B) in every CXT LV Cartridge.
13. Open the door of the CleanXtract LV by pressing the 'Door Open' option on the main control interface.
14. Take the prepared CleanNA CXT-LV Cartridge(s) and place them in the machine as shown in Figure 7. **Press until you hear a click.**

⚠ **Note:** The positions on the CleanXtract LV are marked on the deck of the machine, see Figure 7.

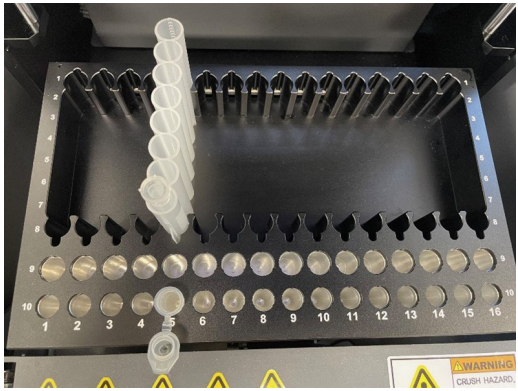


Figure 7: Deck of the CleanXtract LV with position numbers and one set of plastics.

15. Place the CleanNA CXT-LV 1,5 mL Elution tube(s) in position 10 of the machine and open the tube(s), as shown in Figure 3.
16. Close the door of the device by pushing down until it locks (a clicking sound can be heard).

Extraction CleanXtract LV run:

1. To start the CleanXtract LV run, select the 'Run' option on the menu display.
2. A menu will appear in which the type of protocol can be selected. For the purpose of the 1 mL CPCF extraction, select the Cell-free DNA option.
3. A menu will appear showing different cfDNA extraction protocols. Select the 1 mL protocol.
4. Press the "0" button on the menu screen. This will open a keyboard menu in which the number of samples can be manually entered. If the correct number has been entered, press the "OK" button.
5. Two instruction messages will appear on the CleanXtract LV display: one instructing to close the door of the device and the other instructing various sample related matters, see Figure 8. Make sure to follow these instructions thoroughly to prevent an error from occurring.

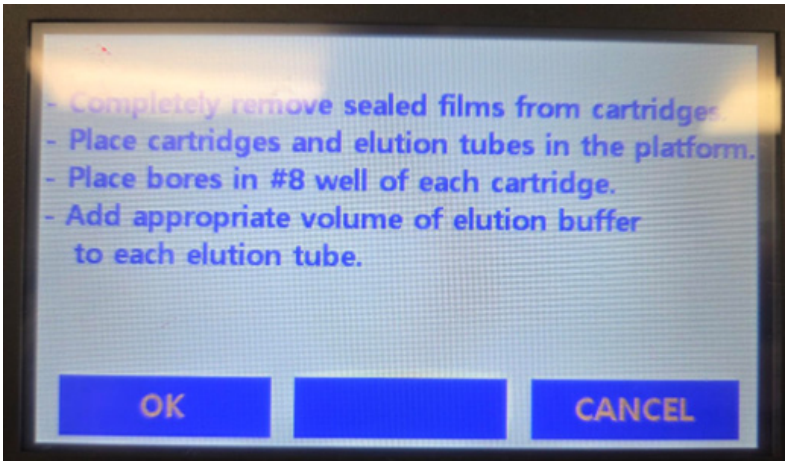


Figure 8: Instructions on the interface of the CleanXtract LV before starting the run.

6. By pressing the "OK" button after following the instructions on the screen, the CleanXtract LV run will start.
7. When necessary, press the red "STOP" button in order to manually stop the run.
8. When a manual stop has been performed, make sure to press the "BORE RELEASE" button on the main display. This will place the bore back into the cartridge well 8.

⚠ **Note:** Skipping this step could damage the device.

9. When the CleanXtract LV run is finished, remove the cartridges and elution tubes containing the purified DNA. Close and switch off the CleanXtract LV.
10. Close the tubes and store the DNA at -20 °C.

2 mL Input Volume - CleanXtract LV

Before Starting:

• Prepare sample samples according to 'Collection, Storage and Handling of Specimen' on page 10.
• Turn on the CleanXtract LV and use the UV function for 20 minutes.
• Set the thermoshaker at 60°C.
• Prepare IG Wash according to the instructions in the Preparation of Reagents section on Page 12.
•Prepare a fresh 80% ethanol solution.
• Make sure the Cell-Free Particles and Proteinase K (20 mg/mL) are at room temperature.
• Shake or vortex the Cell-Free Particles to fully resuspend the particles before pipetting.

Sample Preparation:

1. Add 2 mL sample to 5,0 mL tubes (if the sample volume is less, fill up to 2 mL with Elution Buffer LT).

⚠ **Note:** Do not exceed the maximum sample volume, this will decrease the efficiency of the extraction procedure.

2. Prepare a CPCF lysis mix in a separate tube according to Table 10 and mix by inverting the tube for at least ten times.

Table 10: CPCF Lysis mix preparation for 2 mL sample input.

Reagent	1 Sample	16 Samples*
CFL Buffer	135 µL	2268 µL
Proteinase K	30 µL	504 µL
Total	165 µL	2,8 mL

*Includes 5% excess volume.

3. Add 165 µL of CPCF Lysis mix to each sample.
4. Place the tubes in the pre-heated shaker (60°C) for 25 minutes at 1000 rpm.
5. Take the tubes from the shaker and let them cool down for 10 minutes at room temperature.

6. Prepare the CPCF Binding mix in a separate tube according to Table 7.

Table 7: CPCF Binding mix preparation for 2 mL sample input.

Reagent	1 Sample	16 Samples*
DB Buffer	2000 µL	33,6 mL
CleanNA Particles LV	20 µL	336 µL
Total volume	2020 µL	34 mL

*Includes 5% excess volume

⚠ **Note:** Shake or vortex the CleanNA Particles LV to fully resuspend the particles before use.

7. Position CleanNA CXT LV Cartridge(s) (Figure 9A) in the Cartridge Rack according to the number of sample(s) required, as shown in Figure 9D.

⚠ **Note:** The well with the protrusion is well 8, indicated in the orange circle.

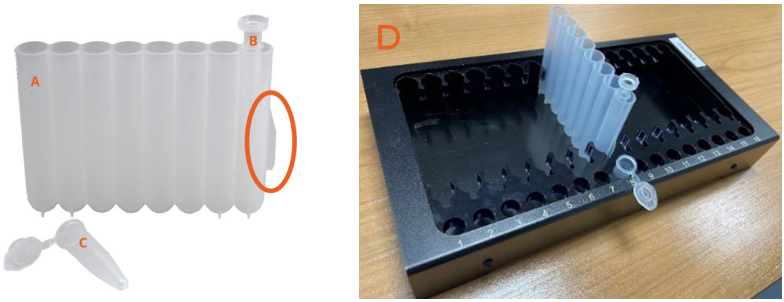
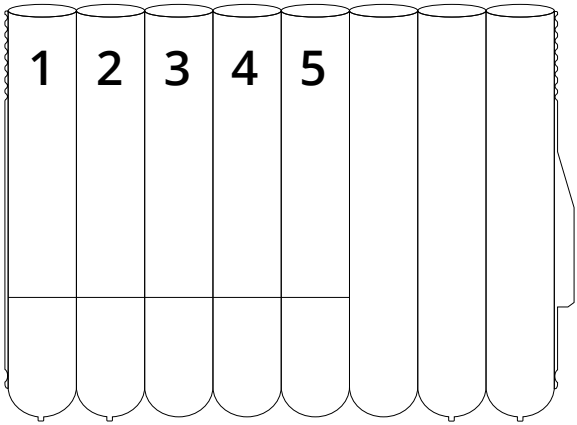


Figure 9: (A) = CleanNA CXT-LV Cartridge (B) = CleanNA CXT-LV Bore, (C) = CleanNA CXT-LV 1,5 mL Elution tube and (D) = Cartridge rack with one set of plastics.

8. Fill the cartridge(s) as mentioned in Table 11 and Figure 10.

Table 11: Plate positions for the CPCF extraction protocol on the CleanXtract LV.

Well number	Reagent used	Volume
1	CPCF Binding mix	1010 µL
2	CPCF Binding mix	1010 µL
3	IG Wash	1000 µL
4	80% ethanol	1000 µL
5	80% ethanol	1000 µL
6	N/A	N/A
7	N/A	N/A
8	N/A	N/A



Fig

⚠ **Note:** Shake or vortex the CPCF Binding mix to fully resuspend the particles before use. Invert the CPCF Binding mix at least ten times prior to use and after 4 samples.

⚠ **Note:** IG Wash must be diluted with Isopropanol prior to use. Please see Page 12 for instructions.

9. After preparing the cartridges, seal them with a plastic seal until the sample needs to be added.
10. Fill the CleanNA CXT-LV 1,5 mL Elution tube(s) (Figure 6C) with 40-80 µL Elution Buffer LT.
11. Transfer **1 mL** of the lysate (Sample + CPCF Lysis mix) to well 1 and **1 mL** lysate to well 2 of the CleanNA CXT-LV Cartridge (this well already contains the CPCF Binding Mix).
12. Place a CleanNA CXT-LV Bore in well 8 (Figure 6B) in every CXT LV Cartridge.
13. Open the door of the CleanXtract LV by pressing the 'Door Open' option on the main control interface.
14. Take the prepared CleanNA CXT-LV Cartridge(s) and place them in the machine as shown in Figure 7. **Press until you hear a click.**

⚠ **Note:** The positions on the CleanXtract LV are marked on the deck of the machine, see Figure 11.

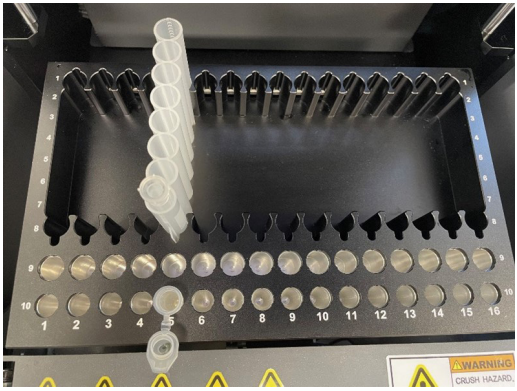


Figure 11: Deck of the CleanXtract LV with position numbers and one set of plastics.

15. Place the CleanNA CXT-LV 1,5 mL Elution tube(s) in position 10 of the machine and open the tube(s), as shown in Figure 3.
16. Close the door of the device by pushing down until it locks (a clicking sound can be heard).

Extraction CleanXtract LV run:

1. To start the CleanXtract LV run, select the 'Run' option on the menu display.
2. A menu will appear in which three types of protocols can be selected. For the purpose of the 2 mL CPCF extraction, select the Cell-free DNA option.
3. Another menu will appear showing different cfDNA extraction protocols. Select the 2 mL protocol.
4. Press the "0" button on the menu screen. This will open a keyboard menu in which the number of samples can be manually entered. If the correct number has been entered, press the "OK" button.
5. Two instruction messages will appear on the CleanXtract LV display: one instructing to close the door of the device and the other instructing various sample related matters, see Figure 12. Make sure to follow these instructions thoroughly to prevent an error from occurring.

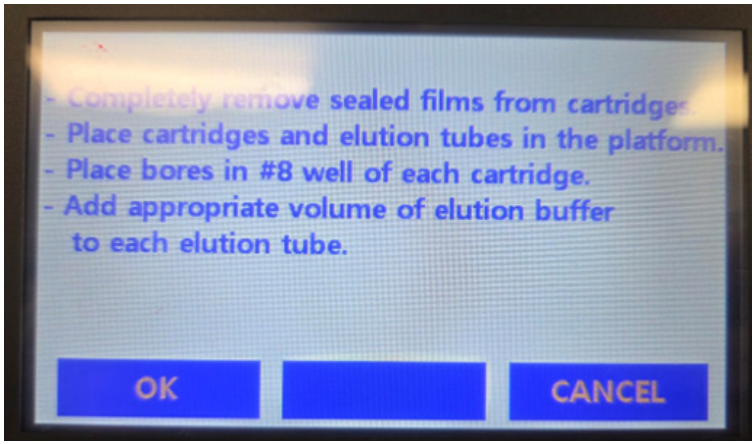


Figure 12: Instructions on the interface of the CleanXtract LV before starting the run.

6. By pressing the "OK" button after following the instructions on the screen, the CleanXtract LV run will start.
7. When necessary, press the red "STOP" button in order to manually stop the run.
8. When a manual stop has been performed, make sure to press the "BORE RELEASE" button on the main display. This will place the bore back into the cartridge well 8.

⚠ **Note:** Skipping this step could damage the device.

9. When the CleanXtract LV run is finished, remove the cartridges and elution tubes containing the purified DNA. Close and switch off the CleanXtract LV.
10. Close the tubes and store the 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 Cell-Free Particles.	Resuspend the Cell-Free Particles by vortexing vigorously before use.
	Inefficient binding of the DNA to the Cell-Free Particles.	Ensure to let the sample cool at room temperature for 10 minutes prior to addition of the DB Buffer.
		Ensure to mix each sample continuously throughout the binding incubation.
	Loss of Cell-Free Particles during operation.	Avoid disturbing the Cell-Free Particles during aspiration.
	DNA remains bound to Cell-Free Particles.	Increase elution volume and incubate at 56 °C temperature for 15 minutes; Pipet up and down 50 to 100 times.
	DNA washed off.	Dilute IG Wash by adding appropriate volume of IPA absolute prior to use (see Page 12 for instructions).
	Ethanol carryover.	Dry the Cell-Free Particles at room temperature for 5-10 minutes longer than stated in the protocol.
High variation in DNA yield	Settling of the cell-free particles.	Vortex the Cell-Free Particles before pipetting every sample.
Cell-Free Particles do not completely clear from solution	Too short magnetizing time.	Increase collection time on the magnetic separation device.
Problems in downstream applications	Salt carryover.	80% ethanol must be at room temperature.
CleanXtract LV did not pickup bore(s)	Cartridges/bores were not placed properly in the CleanXtract LV.	Press Cartridges into the CleanXtract LV until a click is heard.
		Place bores in the correct well (8).

Symbols

IVD	In-vitro Diagnostics
CE	CE mark. This product meets the requirements for CE-IVD device under the EU Regulation for In Vitro Diagnostic Medical Devices (2017/746)
REF	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
LOT	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Plasma cfDNA Kit Dx (96 preps)	CPCF2Dx-D0096

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
1	2026/AUG/06	Initial version	Initial version

Notes

Notes

Contact

Coenecoop 75 | 2741 PH Waddinxveen | The Netherlands
+31 (0) 182 22 33 50 | info@cleanna.com
www.cleanna.com