



Clean Blood & Tissue DNA Kit

Instructions for Use

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For Research Use Only

REF CBT-D0016, CBT-D0096, CBT-D0384

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Intended Purpose

The intended purpose of the Clean Blood & Tissue DNA Kit is to extract DNA from human blood, tissue, buffy coat and cultured cells in a sufficient purity to be used in downstream detection procedures based on the principle of Polymerase Chain Reaction (PCR). It can be used manually or on automated extraction devices that can handle magnetic particles.

Intended User

The Clean Blood & Tissue DNA Kit is intended to be used by professionals who are trained in molecular biology laboratory techniques.

Introduction and Principle

The Clean Blood & Tissue DNA Kit is based upon our proprietary magnetic particle-based system to extract high quality genomic DNA from blood and tissue. It can be used for DNA extraction from 250 μL of human whole blood or 5-50 μL buffy coat containing anticoagulants such as Citrate, EDTA and Heparin, as well as for DNA extraction from up to 10 mg of tissue or 5×10^5 – 5×10^6 cultured cells.

The extraction protocol is fully scalable and, due to the use of our magnetic particle purification technology, can easily be automated on our CleanXtract 96 and general liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™). Alternatively, each protocol can be performed manually. Extracted DNA is suitable for direct use in most downstream applications, such as PCR amplification.

Schematic Overview

The blood or tissue samples are lysed using our **Proteinase K** and specially formulated **lysis buffers**, which are optimized for the various types of starting material. DNA is isolated from the lysates in one step by binding to the surface of the **Blood DNA Particles**. The magnetic particles are separated from the lysates by using a magnetic separation device. Following a few rapid steps with the **wash buffers** to remove traces of contaminants, the purified DNA is eluted from the magnetic particles for downstream applications using our **Elution Buffer**.

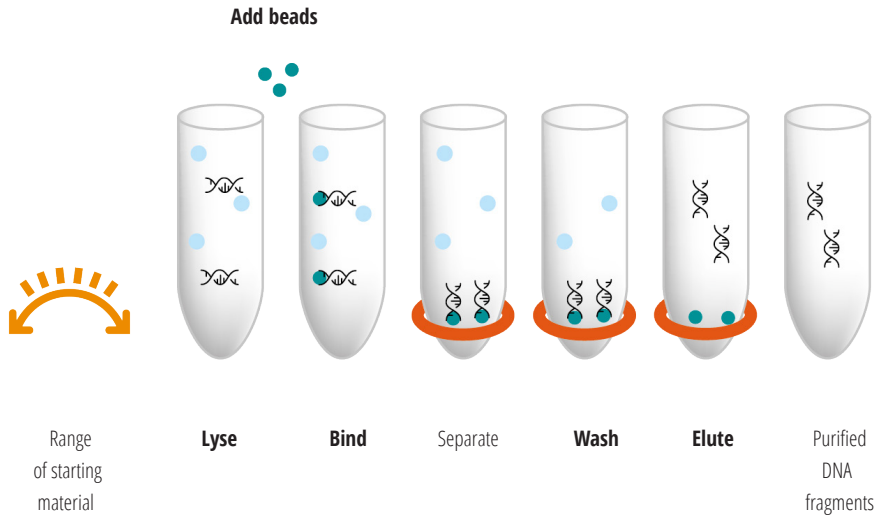


Figure 1: Schematic overview of the Clean Blood & Tissue DNA Kit extraction procedure.

Materials Provided


Kit Contents:

Component	CBT-D0016 (Trial)	CBT-D0096 (96 preps)	CBT-D0384 (384 preps)
TN Lysis	5 mL	30 mL	120 mL
LG Lysis	6 mL	35 mL	135 mL
Blood DNA Particle Solution	5 mL	27 mL	110 mL
GH Wash	11 mL	55 mL	220 mL
TB Wash	6 mL	30 mL	85 mL
Proteinase K (20 mg/mL)	0,35 mL	2,2 mL	9 mL
Elution Buffer	15 mL	60 mL	200 mL

Reagent Shipping, Storage and Handling

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

Component	Storage Temperature
TN Lysis	15-25 °C
LG Lysis	15-25 °C
Blood DNA Particle Solution	2-8 °C
GH Wash	15-25 °C
TB Wash	15-25 °C
Proteinase K (20 mg/mL)	2-8 °C
Elution Buffer	15-25 °C

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

Warnings

Read the instructions carefully before using the kit. Do not mix several kit LOT numbers.

Explanation of LOT number use on the product labels:

- Blood DNA Particle Solution and Proteinase K **boxes** contain the **LOT number of the kit** the component is part of (for traceability).
- Blood DNA Particle Solution 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) by scanning the QR code on the kit box label. You can also request the SDS via www.cleanna.com/sds-request.

TN Lysis



Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. May cause damage to organs through prolonged or repeated exposure. Avoid breathing dust/fume/gas/mist/vapours/spray. Wash skin thoroughly after handling.

IF SWALLOWED: Call doctor or Poisons Information Centre if feeling unwell.

IF ON SKIN: Wash with plenty of water.

IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call doctor or Poisons Information Center if feeling unwell.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

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.

GH Wash



Harmful if swallowed or inhaled. Causes skin irritation. Causes serious eye irritation. May cause damage to organs through prolonged or repeated exposure. Avoid breathing dust/fume/gas/mist/vapours/spray. Wash skin thoroughly after handling.

IF SWALLOWED: Call doctor or Poisons Information Centre if feeling unwell

IF ON SKIN: Wash with plenty of water.

IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call doctor or Poisons Information Center if feeling unwell.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

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

Quality Control

CleanNA produces each lot of Clean Blood & Tissue DNA 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 certified.

Materials and Equipment to be Supplied by User

For manual protocols

General materials and reagents to be supplied by user:

- Absolute ethanol
- 1,5/2,0 mL microcentrifuge tubes
- Magnetic separation device for 1,5/2,0 mL microcentrifuge tubes
- Vortex
- 96-well plate or single tubes for storage of isolated gDNA
- Multichannel pipettes and reagent reservoirs
- Single channel pipettes
- Sealing film
- Thermoshaker capable to be set at 56 °C and 65 °C. If not available, use a waterbath or heatblock

Additional required materials for Buffy Coat and the Cultured Cells protocol:

- 1X PBS

Additional required materials for the Tissue protocol:

- Centrifuge with swing-bucket rotor capable of 3,000 x g
- DTT

For automated protocols

General materials and reagents to be supplied by user:

- CleanXtract 96 (Cat No. CXT-I096)
- 2,2 mL 96 deep-well plate (Cat No. CXT-P096)
- 96-well tip-comb (Cat No. CXT-T096)
- Vortex
- Absolute ethanol
- Sealing film

Additional required materials for Buffy Coat:

- 1X PBS

Additional required materials for the Tissue protocol:

- Centrifuge with swing-bucket rotor capable of 3,000 x g
- DTT

Preparation of Reagents

GH Wash

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

Kit	Absolute ethanol to be added
CBT-D0016	14 mL
CBT-D0096	70 mL
CBT-D0384	280 mL

TB Wash

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

Kit	Absolute ethanol to be added
CBT-D0016	14 mL
CBT-D0096	70 mL
CBT-D0384	198 mL

Manual protocols

Blood or buffy coat Protocol - 250 μ L

The procedure below has been optimized for use with 250 μ L fresh or frozen blood or 5-50 μ L buffy coat samples. This protocol describes the usage of single 1,5 or 2,0 mL tubes with a compatible magnet stand.

Before Starting:

- Prepare all reagents according to the “Preparation of Reagents” section on Page 11.
- Set a thermoshaker to 56 °C.

⚠ Note: If a thermoshaker is not available, incubate the tube(s) in an incubator and vortex according to the notes in the protocol. Vortexing may cause partial DNA fragmentation. When high-molecular-weight analytes are required, the use of a vortex mixer is not recommended.

- Make sure all reagents are at room temperature before use.
- Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.
- Optionally, set a heat block, incubator or water bath to 65 °C.

Protocol:

1. Add **up to 250 μ L blood** or **5-50 μ L buffy coat** sample to 1,5/2,0 mL tubes. Fill up to 250 μ L with Elution buffer (for blood) or PBS (for buffy coat).

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

2. Add 20 μ L Proteinase K (20 mg/mL) to each sample. Pipet up and down 15 times to mix.
3. Add 290 μ L LG Lysis to each sample and pipet up and down 15 times to mix.
4. Incubate the tubes for 10 minutes at 56 °C in a thermoshaker.


⚠ Note: When using an incubator, vortex every 2-3 minutes.

5. Add 250 μ L Blood DNA Particle Solution to each sample.

⚠ Note: Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles before use.

6. Incubate at room temperature for 10 minutes and invert every 2 minutes.

- Place the tubes on a magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature for at least 5 minutes, until the Blood DNA Particles are completely cleared from solution.


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

- Aspirate and discard the cleared supernatant.

Note: Do not disturb the Blood DNA Particles.

- Remove the tubes from the magnetic separation device.


- Add 600 μ L GH Wash to each sample.

 **Note:** GH Wash must be diluted with ethanol prior to use. Please see Page 11 for instructions.

- Resuspend the Blood DNA Particles by pipetting up and down 15 times.

 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity.


- Place the tubes on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.

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

- Aspirate and discard the cleared supernatant. Do not disturb the Blood DNA Particles.

- Remove the tubes from the magnetic separation device.


- Add 600 μ L TB Wash to each sample.

 **Note:** TB Wash must be diluted with ethanol prior to use. Please see Page 11 for instructions.

- Resuspend the Blood DNA Particles by pipetting up and down 15 times.


 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity.

- Place the plate on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.


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

- Aspirate and discard the cleared supernatant. Do not disturb the Blood DNA Particles.

19. Leave the tubes on the magnetic separation device and wait for 1 minute.
20. Remove any residual liquid from the wells using a pipet.
21. While leaving the tubes on the magnet, dry the Blood DNA Particles for at least 5 minutes.
22. Remove the tubes from the magnetic separation device.
23. Add 100 μ L Elution Buffer to elute DNA from the Blood DNA Particles.

 **Note:** Make sure the elution buffer covers the Blood DNA Particles. Too low elution volumes can cause lower yield. Too high volumes cause lower concentration of DNA in the eluate.

24. Resuspend the particles by using a pipette and incubate for 10 minutes.

 **Note:** Incubating at 65°C during elution can increase yield.

25. Place the plate on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.
26. Transfer the cleared supernatant containing purified DNA to a clean microplate or tube (not supplied). Store the DNA at -20°C.

Tissue Protocol - up to 10 mg

The procedure below has been optimized for use with up to 10 mg tissue. This protocol describes the usage of single 1,5 or 2,0 mL tubes with a compatible magnet stand.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 12.
- Set a thermoshaker to 56°C.

Note: If a thermoshaker is not available, incubate the tube in an incubator and vortex according to the notes in the protocol. Vortexing may cause partial DNA fragmentation. When high-molecular-weight analytes are required, the use of a vortex mixer is not recommended.

- Make sure all reagents are at room temperature before use.
- Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.
- Optionally, set a heat block, incubator or water bath to 65 °C.

Protocol:

1. Mince up to 10 mg of tissue into a tube. Do not exceed sample weight.
2. Create a TN Lysis/DTT master mix as follows:
 - The required final DTT concentration is 40 mM in TN Lysis.
 - To prepare directly before use, add 40 µL 1 M DTT per 1 mL TN Lysis and use immediately.
3. Add 250 µL Lysis master mix per sample.

Note: Cut the tissue into small pieces to speed up lysis.


4. Add 20 µL Proteinase K (20 mg/mL). Pipet up and down 15 times to mix thoroughly.
5. Incubate at 56°C for 1-3 hours in a thermoshaker.

Note: When using an incubator, vortex every 20-30 minutes.

6. Centrifuge at 3,000 x g for 5 minutes at room temperature.
7. Transfer 200 µL cleared lysate into a new tube and continue to step 8 using the cleared lysate.
8. Add 230 µL LG Lysis to each sample and pipet up and down 15 times to mix.
9. Incubate the tubes at 56 °C for 10 minutes in a thermoshaker.


Note: When using an incubator, vortex every 2-3 minutes.

10. Add 200 μ L Blood DNA Particle Solution to each sample.

 **Note:** Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles before use.

11. Incubate at room temperature for 10 minutes and invert every 2 minutes.

12. Place the tube on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the 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 the Blood DNA Particles.

14. Remove the tube containing the Blood DNA Particles from the magnetic separation device.


15. Add 600 μ L GH Wash to each sample.

 **Note:** GH Wash must be diluted with ethanol prior to use. Please see Page 12 for instructions.

16. Resuspend the Blood DNA Particles by pipetting up and down 15 times.

 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity.

17. Place the tube on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.


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

18. Aspirate and discard the cleared supernatant. Do not disturb the Blood DNA Particles.

19. Remove the tube containing the Blood DNA Particles from the magnetic separation device.

20. Repeat Steps 15-19 once for a second GH Wash wash step.


21. Add 600 μ L TB Wash to each sample.

 **Note:** TB Wash must be diluted with ethanol prior to use. Please see Page 12 for instructions.


22. Resuspend the Blood DNA Particles by pipetting up and down 15 times.

 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity.


23. Incubate at room temperature for 1 minute.
24. Place the tube on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.

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

25. Aspirate and discard the cleared supernatant. Do not disturb the Blood DNA Particles.
26. Leave the tube on the magnetic separation device and wait for 1 minute.
27. Remove any residual liquid from the wells using a pipet.
28. While leaving the tube on the magnet, dry the Blood DNA Particles for 10 minutes at room temperature.
29. Remove the tube containing the Blood DNA Particles from the magnetic separation device.
30. Add 100 μ L Elution Buffer to elute DNA from the Blood DNA Particles.

 **Note:** Make sure the elution buffer covers the Blood DNA Particles. Too low elution volumes can cause lower yield. Too high volumes cause lower concentration of DNA in the eluate.

31. Resuspend the particles by using a pipette incubate for 10 minutes.

 **Note:** Incubating at 65°C during elution can increase yield.

32. Place the tube on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the Blood DNA Particles are completely cleared from solution.
33. Transfer the cleared supernatant containing purified DNA to new tubes or a clean microplate (not supplied). Store the DNA at -20°C.

Cultured Cells Protocol

The procedure below has been optimized for use with 5×10^5 – 5×10^6 cultured cells in 250 μ L 1x PBS. This protocol describes the usage of single 1,5 or 2,0 mL tubes with a compatible magnet stand.

Before Starting:

- Prepare all reagents according to the “Preparation of Reagents” section on Page 12.
- Set a thermoshaker to 56 °C.

Note: If a thermoshaker is not available, incubate the tube(s) in an incubator and vortex according to the notes in the protocol. Vortexing may cause partial DNA fragmentation. When high-molecular-weight analytes are required, the use of a vortex mixer is not recommended.

- Make sure all reagents are at room temperature before use.
- Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.
- Optionally, set a heat block, incubator or water bath to 65 °C.

Protocol:

1. Prepare the cell suspension.
 - a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation. Wash the cells with cold PBS (4°C) and resuspend cells in 250 μ L cold PBS. Proceed with Step 2 of this protocol.
 - b. For cells grown in suspension, pellet 5×10^6 cells at 1,200g in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 250 μ L cold PBS. Proceed with Step 2 of this protocol.

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


2. Add 20 μ L Proteinase K (20 mg/mL) to each sample. Pipet up and down 15 times to mix.
3. Add 290 μ L LG Lysis to each sample and pipet up and down 15 times to mix.
4. Incubate the tubes for 10 minutes at 56 °C in a thermoshaker.

Note: When using an incubator, vortex every 2-3 minutes.

5. Add 250 μ L Blood DNA Particle Solution to each sample.

Note: Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles before use.


6. Incubate at room temperature for 10 minutes and invert every 2 minutes.
7. Place the tubes on a magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature for at least 5 minutes, until the Blood DNA Particles are completely cleared from solution.

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


8. Aspirate and discard the cleared supernatant.

Note: Do not disturb the Blood DNA Particles.


9. Remove the tubes from the magnetic separation device.
10. Add 600 μ L GH Wash to each sample.

 **Note:** GH Wash must be diluted with ethanol prior to use. Please see Page 12 for instructions.


11. Resuspend the Blood DNA Particles by pipetting up and down 15 times.

 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity. Due to high DNA concentration the beads can form clumps, this does not influence the purity of the DNA.


12. Place the tubes on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the 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. Do not disturb the Blood DNA Particles.
14. Remove the tubes from the magnetic separation device.
15. Add 600 μ L TB Wash to each sample.

 **Note:** TB Wash must be diluted with ethanol prior to use. Please see Page 12 for instructions.


16. Resuspend the Blood DNA Particles by pipetting up and down 15 times.

 **Note:** Resuspension of the Blood DNA Particles is critical for obtaining good purity. Due to high DNA concentration the beads can form clumps, this does not influence the purity of the DNA.


17. Place the plate on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.

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

18. Aspirate and discard the cleared supernatant. Do not disturb the Blood DNA Particles.
19. Leave the tubes on the magnetic separation device and wait for 1 minute.
20. Remove any residual liquid from the wells using a pipet.
21. While leaving the tubes on the magnet, dry the Blood DNA Particles for at least 5 minutes.
22. Remove the tubes from the magnetic separation device.
23. Add 100 μ L Elution Buffer to elute DNA from the Blood DNA Particles.

 **Note:** Make sure the elution buffer covers the Blood DNA Particles. Too low elution volumes can cause lower yield. Too high volumes cause lower concentration of DNA in the eluate.

24. Resuspend the particles by using a pipette and incubate for 10 minutes at 65 °C.

 **Note:** Incubating at room temperature during elution can lower the yield.

25. Place the plate on the magnetic separation device to magnetize the Blood DNA Particles. Incubate at room temperature until the particles are completely cleared from solution.
26. Transfer the cleared supernatant containing purified DNA to a clean microplate or tube (not supplied). Store the DNA at -20°C.

Automation protocols

Blood or Buffy coat Protocol - CleanXtract 96

Before starting:

- Prepare the wash buffers according to the instructions in the Preparation of Reagents section on Page 11.
- Make sure all reagents are at room temperature before use.
- Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles before use.
- Optional: Perform a UV-decontamination on the CleanXtract 96 before use.

Sample preparation

1. Take a 2,2 mL 96 deep-well plate and name it "2 Lysis/Binding".
2. **For buffy coat only:** add 5-50 μL buffy coat to a 1,5/2,0 mL tube and fill up with Elution Buffer (for blood) or 1x PBS (for buffy coat) to a total volume of 250 μL for each sample. Invert at least 5 times.
3. Add 250 μL blood or the 250 μL buffy coat solution to each well of the 2,2 mL 96 deep-well plate 2 (Lysis/Binding).
4. Prepare a CBT Lysis mix in a separate tube according to Table 1 and mix after by inverting at least ten times.

Table 1: CBT Lysis mix preparation.

Reagent	1 Sample	96 Samples*
LG Lysis	290 μL	29,2 mL
Proteinase K	20 μL	2016 μL

*Includes 5% excess volume

5. Add 310 μL of CBT Lysis mix to each sample.
6. Take another 2,2 mL 96 deep-well plate and name it "1 Tip-comb" and place a tip-comb into the plate.

7. Select “Run setting” in the middle of the CleanXtract 96 display as shown in Figure 2.

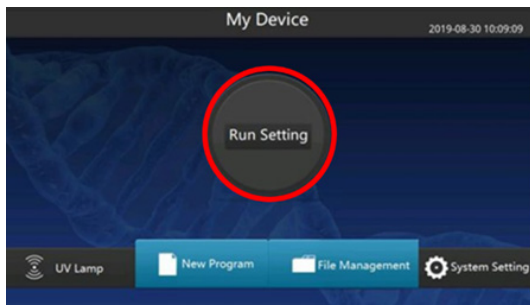


Figure 2 :Main control interface of the CleanXtract 96 with Run Setting shown in the red circle.

8. Now the various positions on the CleanXtract 96 are shown, as indicated in Figure 3.



Figure 3: Select Position interface of the CleanXtract 96 with heated positions indicated in red.

9. Select “Position 2”, to load the 96 deep-well plate with the tip-comb. The CleanXtract 96 will turn the position to the right side of the machine. Open the blue cover to load the plate 1 (Tip-comb), as mentioned in Figure 4.

Note: If the 2,2 mL 96 deep-well plate has trouble clicking into the position, check if the plate is placed properly before applying force. Also check if the A1 position of the plate is in the correct position of the CleanXtract 96, as indicated in Figure 4.

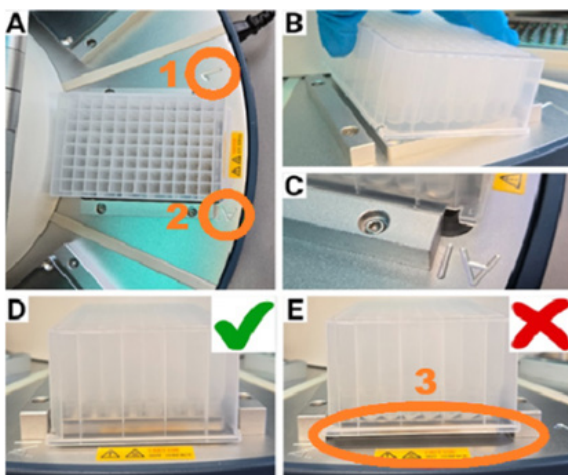


Figure 4: (A) = 2,2 mL 96 deep-well plate placed on the right side of the CXT-96. The plate position of the CXT-96 is indicated with circle 1 and the A1 position is highlighted in circle 2. (B) = How to place the 2,2 mL 96 deep-well plate into the CXT-96. (C) = Notch of the 2,2 mL 96 deep-well plate should be in the correct position. (D) = Correct placement of the 2,2 mL 96 deep-well plate. (E) = Incorrect placement of the 2,2 mL 96 deep-well plate, with the space between the position and plate highlighted with circle 3.

10. Load the plate containing the CBT Lysis mix and sample in the first position. Do this by selecting position 1 on the CleanXtract 96.

Table 2: CleanXtract 96 plate layout for various positions.

Postition	Plate ID	Containing
2	1 (Tip-comb)	Tip-comb
1	2 (Lysis/Binding)	310 μ L CBT Lysis mix + 250 μ L blood sample

11. Close the blue cover of the CleanXtract 96 and press the "<" in the top left corner of the display.
12. Press "File Management" as indicated in the red circle in figure 5.

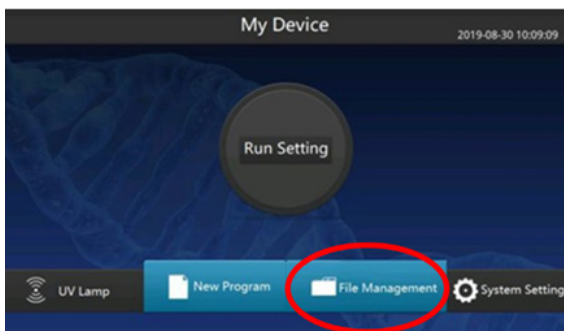


Figure 5: Main control interface of the CleanXtract 96 with File Management indicated in the red circle.

13. Select the “CBT” folder and open the “CXT96_CBT_BL250_LS” protocol.
14. Select “Open.”
15. The protocol will open, and it will show all the steps.
16. Now select “Prepare for Running.”
17. Check if the tip-comb is installed and press “Yes” if the tip-comb is installed.

Note: If the tip-comb is not installed, go back to the main screen and install the tip-comb.

18. The protocol will now start.
19. In the meantime, take four 2,2 mL 96 deep-well plates and fill them according to table below for each sample.

Table 3: Different plates with reagents needed for a Clean Blood & Tissue run.

Plate ID	Reagent used	Volume
3 (Wash 1)	GH Wash	600 µL
4 (Wash 2)	TB Wash	600 µL
5 (Elution)	Elution Buffer	100 µL

20. After the run is finished, take the lysis/binding plate out of the machine.
21. Add 250 µL Blood DNA Particle Soluton to plate 2 (Sample Plate) to each sample.

Note: Shake or vortex the Beads to fully resuspend the particles before use.


22. Take out the plate with the tip-comb and place it in position 1 (according table 4).

Note: Ensure that the 2,2 mL 96 deep-well plate is properly positioned. When correctly placed, a click will be heard. Please check that the plate is in the correct position before applying force. Also check if the A1 position of the plate is in the correct side of the CleanXtract 96, as indicated in Figure 4.

23. Load all the plates in the CleanXtract 96 as indicated in Table 4.

Table 4: CleanXtract 96 plate layout for the various positions.

Position	Plate ID	Containing
1	1 (Tip-comb)	Tip-comb
2	2 (Lysis/Binding)	560 µL lysate + 250 µL Blood DNA Particle Solution
3	3 (Wash 1)	600 µL GH Wash
4	4 (Wash 2)	600 µL TB Wash
5	5 (Elution)	50-100 µL Elution Buffer

24. Close the blue cover of the CleanXtract 96 and press the “<” in the top left corner of the display.
 25. Press “File Management”.
 26. Select the “CBT” folder and open one of the following protocols:
 - CXT96_CBT_BL250_EX
 27. Select “Open.”
 28. The protocol will open, and it will show all the steps.
 29. Now select “Prepare for Running.”
 30. Check if the tip-comb is installed and press “Yes” if the tip-comb is installed.
-  **Note:** If the tip-comb is not installed, go back to the main screen and install the tip-comb.
31. The protocol will now start.
 32. After the run is finished, store the DNA at -20°C.


10 mg Tissue Protocol - CleanXtract 96

Before starting:

- Prepare the wash buffers according to the instructions in the Preparation of Reagents section on Page 11.
- Make sure all reagents are at room temperature before use.
- Shake or vortex the Blood DNA Particle Solution to fully resuspend the particles before use.
- Optional: Perform a UV-decontamination on the CleanXtract 96 before use.

Sample preparation

1. Mince up to 10 mg of tissue into a tube. Do not exceed sample weight.
2. Create a TN Lysis/DTT master mix as follows:
 - The required final DTT concentration is 40 mM in TN Lysis.
 - To prepare directly before use, add 40 μL 1 M DTT per 1 mL TN Lysis and use immediately.
3. Add 250 μL Lysis master mix per sample.

 **Note:** Cut the tissue into small pieces to speed up lysis.

4. Prepare a CBT Tissue Lysis mix according to table 5.

Table 5: CBT Tissue Lysis mix preparation.

Reagent	1 Sample	96 Samples*
TN Lysis/DTT master mix	250 μL	25,2 mL
Proteinase K	20 μL	2016 μL

*Includes 5% excess volume

5. Take another 2,2 mL 96 deep-well plate and name it "1 Tip-comb" and place a tip-comb into the plate.
6. Select "Run setting" in the middle of the CleanXtract 96 display as shown in Figure 6.

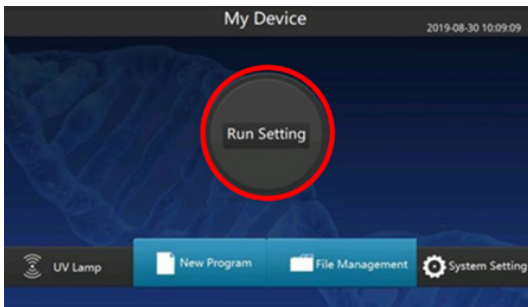


Figure 6 :Main control interface of the CleanXtract 96 with Run Setting shown in the red circle.

7. Now the various positions on the CleanXtract 96 are shown, as indicated in Figure 7.



Figure 7: Select Position interface of the CleanXtract 96 with heated positions indicated in red.

8. Select “Position 2”, to load the 96 deep-well plate with the tip-comb. The CleanXtract 96 will turn the position to the right side of the machine. Open the blue cover to load the plate 1 (Tip-comb), as mentioned in Figure 8.

Note: If the 2,2 mL 96 deep-well plate has trouble clicking into the position, check if the plate is placed properly before applying force. Also check if the A1 position of the plate is in the correct position of the CleanXtract 96, as indicated in Figure 8.

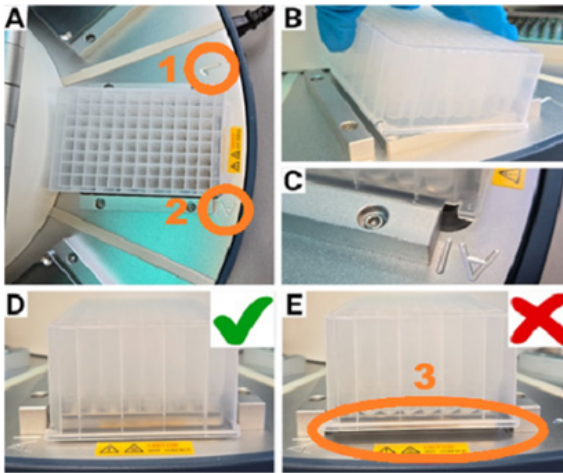


Figure 8: (A) = 2,2 mL 96 deep-well plate placed on the right side of the CXT-96. The plate position of the CXT-96 is indicated with circle 1 and the A1 position is highlighted in circle 2. (B) = How to place the 2,2 mL 96 deep-well plate into the CXT-96. (C) = Notch of the 2,2 mL 96 deep-well plate should be in the correct position. (D) = Correct placement of the 2,2 mL 96 deep-well plate. (E) = Incorrect placement of the 2,2 mL 96 deep-well plate, with the space between the position and plate highlighted with circle 3.

9. Load the plate containing the CBT Lysis mix and sample in the first position. Do this by selecting position 1 on the CleanXtract 96.

Table 6: CleanXtract 96 plate layout for various positions.

Position	Plate ID	Containing
2	1 (Tip-comb)	Tip-comb
1	2 (Tissue Lysis)	CBT Tissue Lysis mix + Tissue sample

10. Close the blue cover of the CleanXtract 96 and press the “<” in the top left corner of the display.
11. Press “File Management” as indicated in the red circle in figure 9.

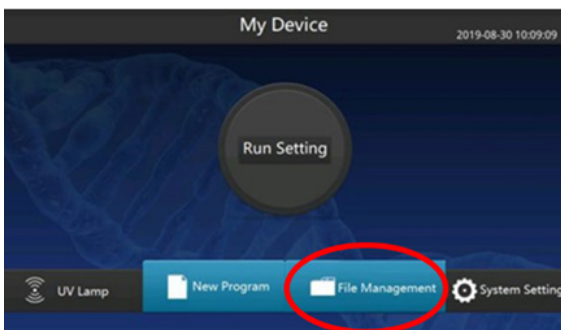


Figure 9: Main control interface of the CleanXtract 96 with File Management indicated in the red circle.

12. Select the “CBT” folder and open the “CXT96_CBT_TS10_LS1” protocol.
13. Select “Open.”
14. The protocol will open, and it will show all the steps.
15. Now select “Prepare for Running.”
16. Check if the tip-comb is installed and press “Yes” if the tip-comb is installed.

Note: If the tip-comb is not installed, go back to the main screen and install the tip-comb.

17. The protocol will now start.
18. After the lysis, take the lysis plate out of the CleanXtract 96. The tip-comb plate can stay in position.
19. Centrifuge the 96 deep-well plate at 3,000 x g for 5 minutes at room temperature.
20. Transfer 200 µL cleared lysate into a new 96 deep-well plate “Sample Plate” and continue to step using the cleared lysate.
21. Add 230 µL LG Lysis to each sample.
22. Load the Sample Plate with LG Lysis in the CleanXtract 96 according to Table 7.

Table 7: CleanXtract 96 plate layout for various positions.

Postition	Plate ID	Containing
2	1 (Tip-comb)	Tip-comb
1	2 (Sample Plate)	230 µL LG Lysis & 200 µL clear lysate

23. Select the “CBT” folder and open the “CXT96_CBT_TS10_LS2” protocol.
24. Select “Open.”
25. The protocol will open, and it will show all the steps.
26. Now select “Prepare for Running.”
27. Check if the tip-comb is installed and press “Yes” if the tip-comb is installed.

Note: If the tip-comb is not installed, go back to the main screen and install the tip-comb.

28. The protocol will now start.
29. In the meantime, take four 2,2 mL 96 deep-well plates and fill them according to table below for each sample.

Table 8: Different plates with reagents needed for a Clean Blood & Tissue run.

Plate ID	Reagent used	Volume
3 (Wash 1)	GH Wash	600 µL
4 (Wash 2)	GH Wash	600 µL
5 (Wash 3)	TB Wash	600 µL
6 (Elution)	Elution buffer	50-100 µL

30. After the run is finished, take the lysis/binding plate out of the machine.

31. Add 200 μ L Blood DNA Particle Solution to plate 2 (Sample Plate) to each sample.

Note: Shake or vortex the Beads to fully resuspend the particles before use.

32. Take out the plate with the tip-comb and replace it in position 1 (according table 9).

Note: Ensure that the 2,2 mL 96 deep-well plate is properly positioned. When correctly placed, a click will be heard. Please check that the plate is in the correct position before applying force. Also check if the A1 position of the plate is in the correct side of the CleanXtract 96, as indicated in Figure 4.

33. Load all the plates in the CleanXtract 96 as indicated in Table 9.

Table 9: CleanXtract 96 plate layout for the various positions.

Position	Plate ID	Containing
1	1 (Tip comb)	Tip-comb
2	2 (Lysis /binding)	200 μ L lysate + 230 μ L LG Lysis + 200 μ L Blood DNA Particle Solution
3	3 (Wash 1)	600 μ L GH Wash
4	4 (Wash 2)	600 μ L GH Wash
5	5 (Wash 3)	600 μ L TB Wash
6	6 (Elution)	50 – 200 μ L Elution Buffer

34. Close the blue cover of the CleanXtract 96 and press the “<” in the top left corner of the display.

35. Press “File Management.”

36. Select the “CBT” folder and open the “CXT96_CBT_TS10_EX” protocol.

37. Select “Open.”

38. The protocol will open, and it will show all the steps.

39. Now select “Prepare for Running.”

40. Check if the tip-comb is installed and press “Yes” if the tip-comb is installed.

Note: If the tip-comb is not installed, go back to the main screen and install the tip-comb.

41. The protocol will now start.

42. After the run is finished, 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 Blood DNA Particle Solution.	Resuspend the Blood DNA Particle Solution by vortexing vigorously before use.
	Loss of Blood DNA Particles during operation.	Avoid disturbing the Blood DNA Particles during aspiration.
	DNA not released from Blood DNA Particles during elution.	Increase elution volume and incubate at 65°C for 15 minutes; pipette up and down 50 to 100 times.
	DNA washed off during wash steps.	Make sure to add ethanol to both GH Wash and TB Wash (see Page 12 for instructions).
	Frozen blood samples not mixed properly after thawing.	Completely thaw the frozen blood at room temperature and gently mix the blood by inverting.
	Inefficient cell lysis due to decrease of activity of the Proteinase K.	Add more Proteinase K (20 mg/mL).
	Inefficient cell lysis due to inefficient mix of Lysis Buffer and Sample.	Ensure the sample is thoroughly mixed with Lysis Buffer.
	Too short of magnetizing time.	Increase collection time on the magnet.
Problems in downstream applications	Salt carryover.	TB Wash must be at room temperature.
Beads do not fully resuspend during wash steps	High DNA concentration causes the beads to clump together	Mix according to the specifications in manual, purity will not be influenced by this
RNA in elution	No RNase step in protocol	Perform RNase step according to the specifications of RNase manufacturer.
After elution sample is too viscous for all the beads to move to the magnet	High DNA concentrations make the elution sample viscous	Increase elution volume to make sample less viscous. Keep the elution samples longer on the magnet until beads are all moved to the magnet

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Blood & Tissue DNA Kit (Trial)	CBT-D0016
Clean Blood & Tissue DNA Kit (96 preps)	CBT-D0096
Clean Blood & Tissue DNA Kit (384 preps)	CBT-D0384

Product	Part Number
Clean Magnet Plate 96-well RN50	CMAG-96-RN50
CleanXtract 96	CXT-I096
2,2 mL 96 deep-well plate	CXT-P096
96-well tip-comb	CXT-T096

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
12	21/JAN/2026	Protocols	Updated text for more clarity and added protocol for cultured cells.
11	27/OCT/2025	Total Revision	Raw materials changed, buffer volumes and names changed, protocols changed.
10	05/MAR/2025	Precautions	Update in hazard symbols.
9	07/JUL/2024	Protocols for Tissue and Saliva	Updated the sentence "Saliva/Tissue samples contain RNA that can purify with the DNA."
8	03/MAY/2024	Total revision.	Updated buffer names.
		Materials provided.	Updated Elution Buffer volume.
		Schematic overview.	Updated picture of workflow.
7	17/OCT/2023	Total revision.	New lay-out. Added chapters schematic overview, warnings, precautions, quality control, symbols.
6	01/OCT/2021	Total revision.	Overall clearer language.
		All protocols.	Included lysis mastermix tables to the protocols.
		Protocols for Buccal swabs, Saliva and Cultured cells.	In mastermix table (step 2 of protocol) changed the unit from μL to ml in the "Total volume for 96-well plate" column.
		Protocol for Mouse Tails Snips.	Added the optional usage of DTT for tough to lyse sample materials.
5	01/AUG/2020	Total revision.	New lay-out.
		User manual information.	General heading before contents added.

Notes

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