CleanPCR

Instructions for Use

V.6 - JUNE 2025

For Research Use Only

REF **CPCR-0001, CPCR-0050, CPCR-0500, CXT-I096**

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Contents

Introduction and Principle

The CleanPCR kit is an efficient PCR and Next Gen library prep clean up system based on paramagnetic particle technology, providing an efficient purification of PCR amplicons. With its simple, three-step protocol, CleanPCR removes salts, primers, primer-dimers, dNTPs, while DNA fragments are selectively bound to the magnetic particles. Highly purified DNA is eluted with low salt elution buffer or molecular biology grade water and can be used directly for downstream applications. The protocol can be adapted to your CleanXtract 96 or liquid handling workstation (Dynamic Devices LYNX™, Hamilton STAR™) utilizing your current protocol, or performed manually.

Features:

- High recovery of amplicons greater than 100 bp
- Efficiently removes unincorporated dNTPs, primers, primer dimers and other contaminants
- Stable and high recovery of PCR products post-cleanup
- No centrifugation or filtration

Amplicons purified with CleanPCR system are ready to be used in the following applications:

- PCR
- Mutation detection and Genotyping
- Sequencing (Sanger and Next Generation)
- Fragment Analysis
- Microarrays
- Restriction enzyme clean up
- Cloning

Materials Provided

Kit Contents:

Product Number	Description	Number of Reactions
CPCR-0001	CleanPCR - 1 mL	55*
CPCR-0050	CleanPCR - 50 mL	2.777*
CPCR-0500	CleanPCR - 500 mL	27.777*

* Number of reactions is based on a typical 10 µL PCR reaction volume. For PCR purification the volume of CleanPCR to be used per reaction = 1.8x the sample volume.

Reagent Shipping, Storage and Handling

Shipping of CleanPCR should be done at room temperature (15-25 °C).

Do not freeze CleanPCR. After CleanPCR has been frozen, it is no longer suitable for use.

Component	Storage Temperature
CleanPCR	2-8 °C

Do not use CleanPCR after the expiration date on the label.

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

Make sure that the kit bottle is not damaged and that no liquid leaked from it. Do not use a kit that has been damaged.

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). Request your SDS via www.cleanna.com/resource-request.

CleanPCR	
No hazard pictogram	No precautionary statement(s) Prevention or Response

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

Quality Control

CleanNA produces each lot of CleanPCR 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

Materials and reagents to be supplied by user for CleanPCR protocols:

- 96-well PCR plate containing PCR samples (up to 50 µL/well)
- Magnetic separation device, recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50)
- (Multichannel) pipettes and tips
- Multichannel Disposable Reservoirs
- 96-well microplate for elution
- 70% ethanol (freshly prepared from non-denatured alcohol)
- Elution Buffer (10mM Tris-HCl pH 8.0 or molecular biology grade water)

For automated protocols

- CleanXtract 96 (Cat No. CXT I096)
- 2,2 mL 96-well DW plate (Cat No. CXT-P096)
- 96-well tip-comb (Cat No. CXT-T096)
- Ethanol absolute
- Nuclease free water

Preparation of Reagents

80% ethanol:
Prepare the 80% ethanol with absolute ethanol and nuclease free water as mentioned on page 9 and store at room temperature. Prepare fresh 80% ethanol before use.

Reagent	1 Sample	96 Samples*
Absolute ethanol	320 µL	32,3 mL
Nuclease free water	80 µL	8,1 mL

* Including 5% excess volume

Manual protocols

96-well Plate Protocol

Before Starting:

- Make sure CleanPCR is at room temperature before starting.
- Thoroughly resuspend the magnetic beads by vortexing.

Protocol:

1. **Shake the CleanPCR reagent thoroughly too fully resuspend the magnetic particles prior to use.**
2. **Measure the PCR sample(s) reaction volume in the wells of the 96-well plate. Determine if transferring the sample(s) to a processing plate is required. If necessary, transfer the PCR reactions to a 96-well microplate.**

⚠ **Note:** If the PCR reaction volume * 2.8 exceeds the volume of the PCR plate, a transfer to a 300 µl round bottom plate is required.

3. Add 1.8x the reaction volume of CleanPCR to each well.

Table 1: Volume ratios for adding CleanPCR in a 96-well plate.

PCR Reaction Volume (µL)	CleanPCR (µL)
10	18
20	36
50	90

4. Pipet up and down 5-10 times or vortex for 30 seconds.
5. Incubate at room temperature for 5 minutes.
6. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.
8. Add 200 µL 70% ethanol to each well.
9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanPCR particles.

10. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR.
11. Repeat Steps 8-10 for a second 70% ethanol wash step.
12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanPCR particles. Remove any residue liquid with a pipette.

⚠ **Note:** It is important to dry the CleanPCR particles before elution. Residual ethanol may interfere with downstream applications.

13. Remove the plate from magnetic separation device.
14. Add 30-40 µL Elution Buffer (not provided) to each well.
15. Pipet up and down 20 times or vortex for 30 seconds.
16. Incubate at room temperature for 2-3 minutes.
17. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR particles are completely cleared from solution.
18. Transfer the cleared supernatant containing purified DNA 96-well microplate and seal with non-permeable sealing film.
19. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

384-well Plate Protocol

Before Starting:

- Make sure CleanPCR is at room temperature before starting.
- Thoroughly resuspend the magnetic beads by vortexing.

Protocol:

1. Shake the CleanPCR reagent thoroughly too fully resuspend the magnetic particles prior to use.
2. Place the 384-well PCR plate on the bench and measure the volume of the PCR reaction. Transfer the sample to a skirted 384-well PCR plate.
3. Add 1.8x the sample volume of CleanPCR reagent to each well.

Table 2: Volume ratios for adding CleanPCR in a 384-well plate.

PCR Reaction Volume (µL)	CleanPCR (µL)
5	9,0
7	12,6
10	18,0

4. Pipet up and down 5-10 times or vortex for 30 seconds.
5. Incubate at room temperature for 5 minutes.
6. Place the plate on a magnetic separation device to magnetize the CleanPCR particles. Incubate at room temperature until the CleanPCR particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.
8. Add 30 µL 70% ethanol to each well.
9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanPCR particles.
10. Aspirate and discard the cleared supernatant. Do not disturb the CleanPCR particles.
11. Repeat Steps 8-10 for a second 70% ethanol wash step.
12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanPCR particles. Remove any residue liquid with a pipette.

⚠ **Note:** It is important to dry the CleanPCR particles before elution. Residual ethanol may interfere with downstream applications.

13. Remove the plate from magnetic separation device.
14. Add 30 µL Elution Buffer (not provided) to each well.
15. Pipet up and down 20 times or vortex for 30 seconds.
16. Incubate at room temperature for 2-3 minutes.
17. Place the plate on a magnetic separation device to magnetize the CleanPCR. Incubate at room temperature until the CleanPCR is completely cleared from solution.
18. Transfer the cleared supernatant containing purified DNA 384-well microplate and seal with non-permeable sealing film.
19. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

Automated protocols

Protocol for 20/25/50 µL input volume

Before starting

1. Shake or vortex the CleanPCR to fully resuspend the particles before use.

Deep-well (DW) plate preparation

1. Prepare 80% ethanol daily according to Table 1.
2. Take a 2,2 mL 96-well DW plate and name it “1 Tip-comb” and place a 96-well tip comb into the DW plate.
3. Get another 2,2 mL 96-well DW plate name it “2 Binding”. Add the amount of sample and CleanPCR together according to Table 3.

Table 3: Different sample input volumes with the corresponding volume of CleanPCR.

Sample volume	Volume CleanPCR
20 µL	36 µL
25 µL	45 µL
50 µL	90 µL

⚠ **Note:** Shake or vortex the CleanPCR to fully resuspend the particles before use.

4. Take three 2,2 mL 96-well DW plates and fill them according to Table 4 for each sample.

Table 4: Different plates with reagents needed for a 20/25/50 µL input CleanPCR run on the CleanXtract 96.

Plate ID	Reagent used	Volume
3 (Wash 1)	80% ethanol	200 µL
4 (Wash 2)	80% ethanol	200 µL
5 (Elution)	Nuclease free water	50 µL

Instrument run

1. Select “Run setting” in the middle of the display.
2. Now the various positions on the CleanXtract 96 are shown, as indicated in figure 1.

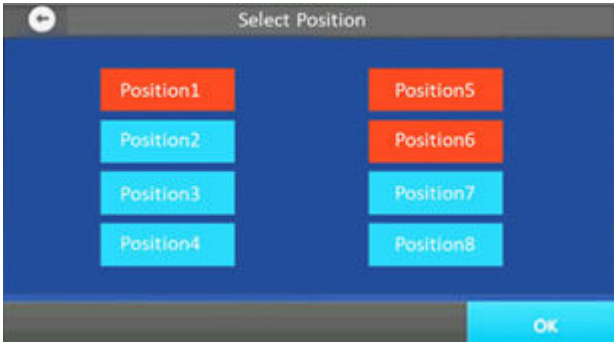

Figure 1: Select Position interface of the CleanXtract 96 with heated positions indicated in red.

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

⚠ **Note:** If the 2,2 mL 96-well DW 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 2.

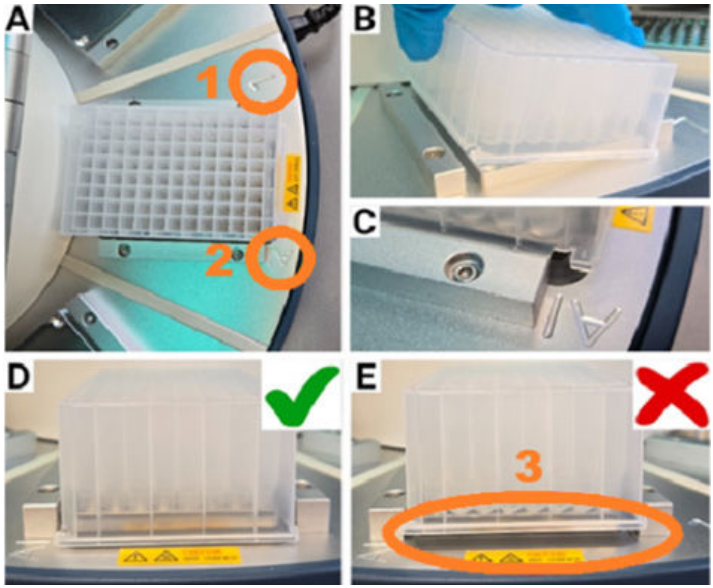

Figure 2: (A) = 2,2 mL 96-well DW plate placed on the right side of the CXT-96. The plate position of the CXT-96 is indicated with circle 1 and the A1 position is highlighted in circle 2. (B) = How to place the 2,2 mL 96-well DW plate into the CXT-96. (C) = Notch of the 2,2 mL 96-well DW plate should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

4. Repeat this process for all the plates as indicated in Table 5.

Table 5: CleanXtract 96 plate layout for the various positions.

Position	Plate ID	Containing
1	1 (Tip-comb)	Tip-comb
2	2 (Binding)	36/45/90 µL CleanPCR & 20/25/50 µL Sample
3	3 (Wash 1)	200 µL 80% ethanol
4	4 (Wash 2)	200 µL 80% ethanol
5	5 (Elution)	50 µL Nuclease free water

5. Close the blue cover of the CleanXtract 96 and press the arrow in the top left corner of the display.
6. Press "File Management" as indicated in the red circle in figure 3.

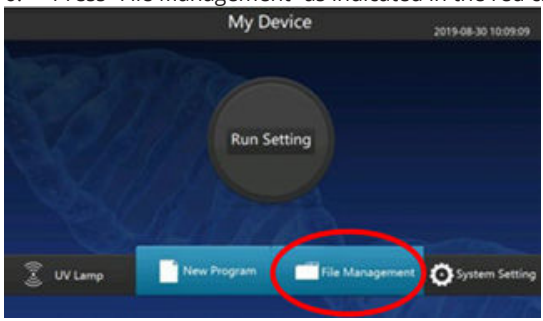

Figure 3: Main control interface of the CleanXtract 96 with File Management indicated in the red circle.

7. Select the "CleanPCR" folder and open the one of the following protocols:
    - CPCR20 96 EX (20 µL sample input)
    - CPCR25 96 EX (25 µL sample input)
    - CPCR50 96 EX (50 µL sample input)
8. Select "Open".
9. The protocol will open, and it will show all the steps.
10. Now select "Prepare for Running".
11. Check and then press "Yes" on the question if the tip-comb is installed.

⚠ **Note:** If the tip-comb is forgotten, go back to the main control interface and repeat step

12. The protocol will now start.
13. 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 yield	Low PCR product yield.	Increase the number amplification cycles for PCR.
	Smaller PCR product size.	Small PCR fragments normally give lower yield.
	Ethanol residue.	During the drying step, remove any liquid from bottom of the well.
	Particle loss during the procedure.	Increase magnetization time. Aspirate slowly.
	DNA remains bound to particles.	Increase elution volume.
	Incomplete resuspension of the particles during elution.	Vortex or pipet up and down to fully resuspend the particles.
Primer carryover	Insufficient wash of the particles.	Wash the particles one more time with 70% ethanol.
Problems in downstream applications	Salt carryover.	70% ethanol must be stored at room temperature.
	Ethanol carryover.	Ensure the particles are completely dried before elution.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

Ordering Information

Contact your local distributor to order.

Product	Part Number
CleanPCR - 1 mL	CPCR-0001
CleanPCR - 50 mL	CPCR-0050
CleanPCR - 500 mL	CPCR-0500

Product	Part Number
Clean Magnet Plate 96-well RN50	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
6	16/JUN/2025	Layout	According to new CleanNA corporate style
		Protocol CleanXtract 96	Added Protocol CleanXtract 96
5	14/MAY/2024	Catalog Numbers	Updated CleanPCR 5 mL details to CleanPCR 1 mL details.
4	01/OCT/2021	Total revision	Improved readability in work process
		CleanPCR 96 well protocol	Changed x.0 µL to x µL.
			added molecular biology grade water as elution buffer.
3	01/NOV/2020	Total revision	New lay-out.
		User manual information	General heading before contents added.

Notes

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