CleanPCR

USER MANUAL

Manual revision v5.00

Catalog Numbers:

CPCR-0001: 1 mL	55 x 10 µL reactions
CPCR-0050: 50 mL	2.777 x 10 µL reactions
CPCR-0500: 500 mL	27.777 x 10 µL reactions

Batch No: See bottle

Shipping: room temperature

Storage and stability: CleanPCR should be stored at 2-8°C upon receipt.

Intended use: CleanPCR is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Quality Control: Each lot of CleanPCR is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at info@cleanna.com or +31 (0) 182 22 33 50.

Safety precautions: When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.

Emergency: In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or info@cleanna.com.

Expiry: When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

FOR RESEARCH USE ONLY

Contents

Introduction and Principle

The 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

Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CPCR-0001	CleanPCR – 1 mL	55 *	2-8°C DO NOT FREEZE
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.

Materials Supplied in the CleanPCR kit:
CleanPCR magnetic particle solution

Materials and Equipment to be supplied by user:

- 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)

CleanPCR - 96-well Plate 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.

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.

CleanPCR - 384-well Plate 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.

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.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible problems and Solutions

Problem	Cause	Solution
Low 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.

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-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
5.00	14/MAY/2024	Catalog Numbers	Updated CleanPCR 5 mL details to CleanPCR 1 mL details.
4.00	October 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
3.00	November 2020	Total revision	New lay-out
		User manual information.	General heading before contents added.