CleanNGS

USER MANUAL

Manual revision v7.00

Catalog Numbers:

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

Batch No: See bottle

Shipping: Room temperature

Storage and stability: CleanNGS should be stored at 4°C upon receipt.

Intended use: CleanNGS 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 CleanNGS 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 CleanNGS kit is an efficient PCR and Next Generation Sequencing Library prep clean up system based on paramagnetic particle technology, providing an efficient purification of PCR amplicons. The CleanNGS kit is manufactured under RNase-free conditions allowing for the purification of RNA and cDNA from invitro applications.
With its simple, three-step protocol, CleanNGS removes salts, primers, primer-dimers, dNTPs, while DNA and/or RNA fragments are selectively bound to the magnetic particles; and highly purified DNA and/or RNA is eluted with low salt elution buffer or molecular biology grade water and can be used directly for downstream applications. The protocol can be performed on a liquid handling workstation (e.g. Dynamic Devices LYNX™, Hamilton STAR™) utilizing a standard protocol, or it can be performed manually.

Features:

- Designed for both DNA and RNA purification
- Ideal for (double-sided) size selection for Next Generation Sequencing
- High recovery of amplicons greater than 100 bp
- Efficiently removes unincorporated dNTPs, primers, primer dimers and other contaminants
- No centrifugation or filtration

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

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

Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CNGS-0001	CleanNGS – 1 mL	55 *	4-8°C DO NOT FREEZE
CNGS-0050	CleanNGS – 50 mL	2.777 *	
CNGS-0500	CleanNGS – 500 mL	27.777 *	

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

Materials Supplied in the CleanNGS kit:

CleanNGS 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
- 80% ethanol (freshly prepared from non-denatured alcohol)
- Molecular biology grade water (RNase free) or Elution Buffer (10mM Tris-HCl pH 8.0)

Working RNase Free

For RNA applications it is important to work RNase free. RNases are present everywhere and general precautions should be taken to avoid the introduction of RNases and other contaminating nucleases while working with RNA. The most common sources of RNases are hands, dust particles and contaminated laboratory solutions, equipment and glassware.

To minimize the risk of RNase contamination we recommend the following precautions:

- Always use gloves when handling RNA samples. Change your gloves frequently, to avoid contaminations;
- Ensure to use RNase free filter tips for pipetting;
- Use materials such as disposable consumables, which are guaranteed RNase free;
- Use reagents which are guaranteed RNase free. Creating aliquots from buffers lowers the risk of RNase contamination in buffers, reagents, etc.;
- Avoid using reagents, consumables and equipment dedicated for common use or general lab processes;
- If possible work in a separate room, fume hood or lab space;
- Clean all working surfaces with commercial RNase inhibiting surfactant or 70% ethanol before starting your work / experiment.

CleanNGS - 96-well Plate Protocol

1. Shake the CleanNGS reagent thoroughly to fully resuspend the magnetic particles prior to use.
2. Measure the 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 reactions to a 96-well microplate.

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

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

PCR Reaction Volume (µL)	CleanNGS (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 CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
8. Add 180 µL 80% ethanol to each well.
9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanNGS particles.
10. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
11. Repeat Steps 8-10 for a second 80% ethanol wash step.
12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanNGS particles. Remove any residue liquid with a pipette.

Note: It is important to dry the CleanNGS 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 CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
18. Transfer the cleared supernatant containing purified DNA and/or RNA to a new (RNase-free) 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.

CleanNGS - 384-well Plate Protocol

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

PCR Reaction Volume (µL)	CleanNGS (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 CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
8. Add 30 µL 80% ethanol to each well.
9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanNGS particles.
10. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
11. Repeat Steps 8-10 for a second 80% ethanol wash step.
12. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanNGS particles. Remove any residue liquid with a pipette.

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

13. Remove the plate from magnetic separation device.
14. Add 20 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 CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
18. Transfer the cleared supernatant containing purified DNA and/or RNA to a new (RNase-free) 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.

CleanNGS - Single Tube Protocol

1. Shake the CleanNGS reagent thoroughly to fully resuspend the magnetic particles prior to use.
2. Measure the volume of the PCR reaction and transfer the sample to a single tube, for example an 1.5 mL single tube.
3. Add 1.8x the sample volume of CleanNGS reagent to each tube.

PCR Reaction Volume (µL)	CleanNGS (µL)
50	90
100	180
150	270

4. Pipet up and down 5-10 times or vortex for 30 seconds.
5. Incubate at room temperature for 5 minutes.
6. Place the tube in the magnetic separation stand to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
8. Remove the tube from the magnetic separation stand.
9. Add 500 - 1000 µL 80% ethanol to each tube.
10. Incubate at room temperature for 1 minute. Briefly resuspend the CleanNGS particles by pipetting up and down.
11. Place the tube in the magnetic separation stand to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
13. Repeat Steps 8-12 for a total of three 80% ethanol wash step.
14. Leave the tube in the magnetic separation stand for 10-15 minutes to air dry the CleanNGS particles. Remove any residue liquid with a pipette.

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

15. Remove the tube from the magnetic separation stand.
16. Add a minimum of 30 µL molecular biology grade water or Elution Buffer (not provided) to each tube.
17. Pipet up and down 20 times or vortex for 30 seconds.
18. Incubate at room temperature for 2-3 minutes.
19. Place the tube in the magnetic separation stand to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
20. Transfer the cleared supernatant containing purified DNA and/or RNA to a new (RNase-free) tube or microplate and seal with non-permeable sealing film.
21. Store the tube(s) or plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be kept at -20°C.

CleanNGS - Double Size Selection protocol (left/right)

Introduction: CleanNGS can be used for double size selection in Next Generation Sequencing (NGS) applications. Typically, library prep kits are provided with a protocol specifying the ratio's (volumes) to be used in order to selectively bind and purify DNA fragments of the desired size (bp).

Binding of larger DNA fragments (right selection): The first addition of CleanNGS will bind DNA fragments larger in size (bp) as the target size. After binding of the DNA to the particles and separation of the CleanNGS particles using a magnet, the supernatant containing the DNA fragments of target size and smaller, will be transferred into a new clean plate.

Binding of desired DNA fragments (left selection): During the second binding step, a second volume of CleanNGS will be added allowing the binding of the target size DNA fragments. Smaller DNA fragments remain in solution, they will be removed and discarded together with the supernatant after particle collection using a magnet.

After some quick ethanol washes, the target size DNA can be eluted from the particles using an elution buffer.

For optimal size selection performance of CleanNGS:
- Sample should contain fragmented double-stranded DNA
- Sample volume should ideally be ≥ 50 µL
- Desired fragment size after size selection should be between 150 and 800 bp
- Left side ratio needs to be greater than the right side ratio

The table below, gives an indication of CleanNGS ratio's to be used allowing the selection and purification of DNA fragments of a specific size range.

bp Region	Ratio used (Left/Right)	Left/Right Selection Delta (bp)
180 -1300	0.90/0.50	1120
200 – 700	0.85/0.56	500
235 – 660	0.80/0.61	425
265 – 575	0.77/0.64	310
280 - 535	0.75/0.67	255

1. Shake the CleanNGS reagent thoroughly to fully resuspend the magnetic particles prior to use.
2. Add the desired volume of CleanNGS to each well.

   Volume of CleanNGS = sample volume * ratio (right)
   Example: CleanNGS volume = 50 µL * 0.7x ratio = 35 µL of CleanNGS

3. Pipet up and down 15-20 times or vortex for 30 seconds.
4. Incubate at room temperature for 5 minutes.
5. Place the plate on a magnetic separation device to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
6. Transfer the clear supernatant, which contains the fragments of the desired size and smaller to a new plate.

7. Add the desired second volume of CleanNGS to each well.

   Volume of CleanNGS = sample volume * (ratio (left) - ratio (right))
   Example: CleanNGS volume = 50 µL * (0.8 - 0.7) = 5 µL of CleanNGS

8. Pipet up and down 15-20 times or vortex for 30 seconds.
9. Incubate at room temperature for 5 minutes.
10. Place the plate on a magnetic separation device to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
11. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
12. Add 180 µL 80% ethanol to each well.
13. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanNGS particles.
14. Aspirate and discard the cleared supernatant. Do not disturb the CleanNGS particles.
15. Repeat Steps 12-14 for a second 80% ethanol wash step.
16. Leave the plate on the magnetic separation device for 10-15 minutes to air dry the CleanNGS particles. Remove any residue liquid with a pipette.

    

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

17. Remove the plate from magnetic separation device.
18. Add 30-40 µL Elution Buffer (not provided) to each well.
19. Pipet up and down 20 times or vortex for 30 seconds.
20. Incubate at room temperature for 2-3 minutes.
21. Place the plate on a magnetic separation device to magnetize the CleanNGS particles. Incubate at room temperature until the CleanNGS particles are completely cleared from solution.
22. Transfer the cleared supernatant containing purified DNA and/or RNA to a new (RNase-free) 96-well microplate and seal with non-permeable sealing film.
23. 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	Inefficient PCR reaction.	Increase the number amplification cycles for PCR.
	Smaller product size (bp).	Small DNA/RNA 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 and/or RNA remains bound to particles.	Prevent over drying the particles and/or increase elution volume.
	Incomplete resuspension of the particles during elution.	Vortex or pipet up and down to fully resuspend the particles.
	RNA degradation.	Ensure to work RNase-free, to prevent RNA loss.
Primer carryover	Insufficient wash of the particles.	Wash the particles one more time with 80% ethanol.
Non-specific amplification products were not removed	The size of the non- specific amplification products are larger than 100 bp.	Non-specific amplification products larger than 100 bp are not efficiently removed from PCR products in the standard protocol (1,8 ratio). Optimization of the CleanNGS versus sample ratio might be required.
Double Size Selection does not give the expected DNA fragment size	Selected DNA fragments are too small (bp).	The ratio of CleanNGS vs sample volume was too high. Try adding less CleanNGS during the size selection process to obtain larger DNA fragments (bp).
	Selected DNA fragments are too large (bp).	The ratio of CleanNGS vs sample volume was too low. Try adding more CleanNGS during the size selection process to obtain larger DNA fragments (bp).
	Contamination of larger DNA fragments after size selection.	Caused by particle carry over from the first binding to the second. Avoid transferring particles after the first binding step.
Problems in downstream applications	Salt carryover.	80% 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
CleanNGS (1 mL)	CNGS-0001
CleanNGS (50 mL)	CNGS-0050
CleanNGS (500 mL)	CNGS-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
7.00	October 2021	Total revision.	CleanNGS (5 mL); CNGS-0005, was replaced by CleanNGS (1 mL); CNGS-0001 on pages 1, 2 and 3. Tube protocol, resuspension of particles during washing was added. Minor text changed increasing readability.
6.00	November 2020	Troubleshooting Guide.	Adjusted Ethanol concentration from 70% to 80%.
5.00	August 2020	User manual general information.	'Notes' added. 'Quality Control Procedure' added.
4.00	August 2020	Total revision.	New lay-out. Important information added at page 1 (before contents). Addition of double size selection protocol. Addition of double size selection troubleshooting guidelines.

Notes

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