Manual CleanNGS

Manual revision v5.00

Catalog Numbers:

CNGS-0005: 5 mL	277 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 biological techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Notes: This reagent has been manufactured under conditions conforming ISO 13485:2016 Quality Management System controls for research and/or further manufacturing.

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.

Quality Control Procedure: please contact us at +31 (0) 182 22 33 50 or info@cleanna.com for the most up-to-date Quality Control Procedure.

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.

Contents

For Research Use Only. Not for use in diagnostic procedures.
Information in this document can subject to change without notice.

Introduction and Principle

The CleanNGS kit is an efficient PCR and Next Gen library prep clean up system based on paramagnetic beads 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 water and can be used directly for downstream applications. The protocol can be adapted to your current liquid handling workstation (e.g. Beckman, Hamilton, Tecan, Caliper, Perkin Elmer, Agilent and Eppendorf) utilizing your current protocol as well as 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:

- PCR[1)] and RT-PCR
- Mutation detection and Genotyping
- Sequencing (Sanger and Next Generation)
- Fragment Analysis
- Microarrays
- Restriction enzyme clean up
- Cloning
- Transfection for RNAi experiments

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Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CNGS-0005	CleanNGS – 5 mL	277 *	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)
- Reagent grade water, RNase free or Elution Buffer (10mM TRIS-HCL pH 8.0)

Working RNase Free

For usage of CleanNGS in 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 CleanNGS.
The most common sources of RNase while working with RNA samples 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 and available, 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.

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CleanNGS - 96-well Plate Protocol

1. Shake the CleanNGS reagent thoroughly too fully resuspend the magnetic particles prior to usage.
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 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 is 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.
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.

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CleanNGS - 384-well Plate Protocol

1. Shake the CleanNGS reagent thoroughly too fully resuspend the magnetic particles prior to usage.
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
7	12.6
10	18

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. Incubate at room temperature until the CleanNGS is 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.

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CleanNGS - Single Tube Protocol

1. Shake the CleanNGS reagent thoroughly too fully resuspend the magnetic particles prior to usage.
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. Add 500 - 1000 µL 80% ethanol to each tube.
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 total of three 80% ethanol wash step.
12. 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.

13. Remove the tube from the magnetic separation stand.
14. Add a minimum of 30 µL RNase-free water or Elution Buffer (not provided) to each tube.
15. Pipet up and down 20 times or vortex for 30 seconds.
16. Incubate at room temperature for 2-3 minutes.
17. Place the tube in the magnetic separation stand to magnetize the CleanNGS. Incubate at room temperature until the CleanNGS is completely cleared from solution.
18. 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.
19. 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.

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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 beads and separation of the CleanNGS beads 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 bead collection using a magnet.

After some quick ethanol washes, the target size DNA can be eluted from the beads 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 between 150 and 800 bp
- Left size 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 too fully resuspend the magnetic particles prior to usage.
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 10-15 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 is completely cleared from solution.
6. Transfer the clear supernatant, which containing the fragments of the desired size and smaller to a new plate.

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7. Add the desired 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 10-15 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 is 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.
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.

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Trouble Shooting 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 yield	Increase the number amplification cycles for PCR
	Smaller product size	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 beads	Increase elution volume
	Incomplete resuspension of the particles during elution	Vortex or pipet up and down to fully resuspend the particles.
	Low RNA recovery	Ensure to work RNase-free, to prevent RNA loss
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with 70% 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.
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 bead carry over from the first binding to the second. Avoid transferring beads after the first binding step.
Problems in downstream applications	Salt carryover	70% ethanol must be stored at room temperature.
	Ethanol carryover	Ensure the beads are completely dried before elution.

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Ordering Information

Contact your local distributor to order.

Product	Part Number
CleanNGS (5 mL)	CNGS-0005
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	Revised Chapter	Date of revision	Explanation of revision
4.00	Total revision	August 2020	• New lay-out • Important information added at page 1 (before contents) • Addition of double size selection protocol • Addition of double size selection troubleshooting guidelines
5.00			• 'Notes' added at section before contents • 'Quality Control Procedure' added at section before contents

Minor changes were made in previous versions.

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Notes

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