CleanNGS

Catalog Nos. CNGS-0005, CNGS-0050, CNGS-0500
Manual revision v3.03

Contents

Introduction and Principle 2
Kit Contents and Materials 3
Working RNase Free 3
CleanNGS - 96-well Plate Protocol 4
CleanNGS - 384-well Plate Protocol 5
CleanNGS – Single Tube Protocol 6
Trouble Shooting Guide 7
Ordering Information 7

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

CLEANNA DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTEND ALLOWED BY LAW, IN NO EVENT SHALL CLEANNA BE LIABLE, WHETER CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTAL DAMAGES IN CONNECTION WITH OR ARRISING FROM THIS DOCUMENT, INCLUDING, BUT NOT LIMITED TO THE USE THEREOF, WHETER OR NOT FORSEEABLE AND WHETER OR NOT CLEANNA IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

TRADEMARKS
The Trademarks mentioned herein are the property of their respective owners.

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

[1)] The PCR Process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffmann-La Roche, Ltd.

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

Kit Contents and Materials

Kit Contents:

Product Number	Description	Number of Reactions	Storage Conditions
CNGS-0005	CleanNGS – 5 mL	277 *	4-8^{0}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.
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 pipettor
- Multichannel Disposable Reservoirs
- 96-well microplate for elution
- 70% 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

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

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 200 µL 70% 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 70% 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 pipet.

    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.

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

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 70% 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 70% 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 pipet.

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.

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

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 70% 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 70% 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 pipet.

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.

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

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 Suggestions

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.
Problems in downstream applications	Salt carryover	70% ethanol must be stored at room temperature.
	Ethanol carryover	Ensure the beads are completely dried before elution.

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

Phone: +31 182 22 33 50 | Web: www.cleanna.com | E-mail: info@cleanna.com

CLEAN
NA