# CleanNGS Dx

## Instructions for Use

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CE IVD

REF CNGSDx-0050, CNGSDx-0500

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# Contents

# Intended Purpose

The intended purpose of the device is cleanup of nucleic acid fragments from complex reaction mixtures like PCR reactions or Next Generation sequencing libraries, and nucleic acid size selection for fragment library preparations to be used in downstream Next Generation Sequencing analysis.

# Intended User

Laboratory employee who is trained in the use of molecular biology laboratory techniques.

# Introduction and Principle

The CleanNGS Dx is an efficient PCR and Next Generation Sequencing Library prep cleanup system based on paramagnetic particle technology, providing an efficient purification of PCR amplicons. The CleanNGS Dx kit is manufactured under RNase-free conditions, allowing for the purification of RNA and cDNA from in vitro applications. Due to its CE-IVD compliance, CleanNGS Dx is suitable for use in in vitro diagnostics.

With its simple bind-wash-elute protocol, CleanNGS Dx removes salts, primers, primer-dimers, dNTPs, while DNA and/or RNA fragments are selectively bound to the magnetic particles. Both single and double size selection can be performed with CleanNGS Dx. Highly purified DNA and/or RNA is eluted with low salt elution buffer or molecular biology grade water and can be used directly for Next Generation Sequencing applications.

Features:
- Designed for both DNA and RNA purification
- Ideal for (double) 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
- For in vitro diagnostic use

# Schematic Overview

For double size selection, CleanNGS Dx is added in a certain volume ratio. The large DNA or RNA fragments bind to the magnetic beads, after which they are separated with a magnetic separation device. The supernatant is transferred and more CleanNGS Dx is added. Now, the beads will bind the fragments of interest. The small fragments and inhibitors will be washed away. After two washing steps, the purified DNA or RNA fragments are eluted from the magnetic beads.

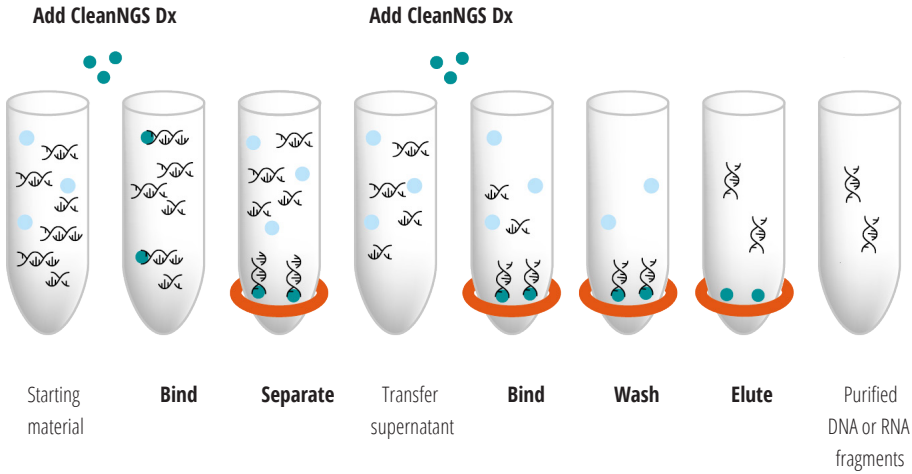


**Figure 1:** Schematic overview of a double size selection CleanNGS Dx procedure.

# Materials Provided

## Kit Contents:

| Product Number | Description | Number of Reactions |
|---|---|---|
| CNGSDx-0050 | CleanNGS Dx - 50 mL | 2.777* |
| CNGSDx-0500 | CleanNGS Dx - 500 mL | 27.777* |

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

# Reagent Shipping, Storage and Handling

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

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

| Component | Storage |
|---|---|
| CleanNGS Dx | 2-8°C |

In use stability: After opening the CleanNGS Dx bottle, the product can be used safely for a period of 63 days.

Do not use CleanNGS Dx 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 bottles are not damaged and that no liquid leaked from the bottles. Do not use a kit that has been damaged.

Any serious incident that has occured in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

# 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/sds-request.

| CleanNGS Dx | |
|---|---|
| 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 CleanNGS Dx 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.

# Limitations

The performance of CleanNGS Dx has been established in combination with the Library Prep Kit, Sequencer and Sequencing Chemistry below:

Library Prep kit:

- Illumina Nextera DNA Flex Library Prep

Sequencer:

- Illumina MiSeq

Sequencing Chemistry:

- Illumina MiSeq Nano v2 PE150 Sequencing Kit

It is the user's responsibility to validate the CleanNGS Dx for other procedures that are not covered by CleanNA's performance evaluation studies.

We recommend the use of an internal extraction control to identify malfunctioning of the device.

Diagnostic results generated after using the CleanNGS Dx must be interpreted in conjuction with other clinical or laboratory findings.

# Collection and Storage of Specimen

It is the responsibility of the user to follow the manufacturers' instructions regarding the use of a Library Prep Kit or other DNA preparation methods.

# Materials and Equipment to be Supplied by User

**Materials and reagents to be supplied by user for the 96-well protocol with CleanNGS Dx:**

- 96-well PCR plate containing NGS or PCR samples (up to 50 µL/well), compatible with the magnetic separation device used
- 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 Dx - 96-well Plate Protocol

## Protocol:

1. Shake the CleanNGS Dx 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 Dx to each well.

| PCR Reaction Volume (µL) | CleanNGS Dx (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 Dx particles. Incubate at room temperature until the CleanNGS Dx particles are completely cleared from solution.
7. Aspirate and discard the cleared supernatant.

⚠ **Note:** Do not disturb or pipet CleanNGS Dx magnetic particles. This can cause lower yield.

8. Add 180 µL freshly made 80% ethanol to each well.
9. Incubate at room temperature for 1 minute. It is not necessary to resuspend the CleanNGS Dx particles.
10. Aspirate and discard the cleared supernatant.

⚠ **Note:** Do not disturb or pipet CleanNGS Dx magnetic particles. This can cause lower yield.

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 Dx particles. Remove any residue liquid with a pipette.

⚠ **Note:** It is important to dry the CleanNGS Dx particles before elution. Residual ethanol may interfere with downstream applications. Do not overdry.

13. Remove the plate from magnetic separation device.

14. Add 30-40 µL molecular biology grade water or Elution Buffer (not provided) to each well.

⚠ **Note:** Using less than the recommended elution volume can cause loss of yield.

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 Dx particles. Incubate at room temperature until the CleanNGS Dx 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.

⚠ **Note:** Take care that no CleanNGS Dx magnetic particles are carried over to the eluate, this may cause interference with downstream applications.

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 Dx - Double Size Selection Protocol (left/right)

**Introduction:** CleanNGS Dx 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 Dx 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 Dxparticles 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 Dx 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 Dx:**
- 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 Dx 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 |
| 220-530 | 0.80/0.70 | 310 |
| 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 Dx reagent thoroughly to fully resuspend the magnetic particles prior to use.
2. Add the desired volume of CleanNGS Dx to each well.
   Volume of CleanNGS Dx= sample volume * ratio (right)
   Example: CleanNGS Dx 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 Dx particles. Incubate at room temperature until the CleanNGS Dx 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.

⚠ **Note:** Do not disturb or pipet CleanNGS Dx magnetic particles. This can cause lower yield.

7. Add the desired second volume of CleanNGS Dx to each well.
   Volume of CleanNGS Dx = sample volume * (ratio (left) - ratio (right))
   Example: CleanNGS Dx 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 Dx particles. Incubate at room temperature until the CleanNGS Dx particles are completely cleared from solution.
11. Aspirate and discard the cleared supernatant.

⚠ **Note:** Do not disturb or pipet CleanNGS Dx magnetic particles. This can cause lower yield.

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 Dx particles.
14. Aspirate and discard the cleared supernatant.

⚠ **Note:** Do not disturb or pipet CleanNGS Dx magnetic particles. This can cause lower yield.

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 Dx particles. Remove any residue liquid with a pipette.

⚠ **Note:** It is important to dry the CleanNGS Dx particles before elution. Residual ethanol may interfere with downstream applications. Do not overdry.

17. Remove the plate from magnetic separation device.
18. Add 30-40 µL molecular biology grade water or Elution Buffer (not provided) to each well.

⚠ **Note:** Using less than the recommended elution volume can cause loss of yield.

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 Dx particles. Incubate at room temperature until the CleanNGS Dx 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.

⚠ **Note:** Do Take care that no CleanNGS Dx magnetic particles are carried over to the eluate, this may cause interference with downstream applications.

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.

# Symbols

| | |
|---|---|
| IVD | In-vitro Diagnostics |
| CE | CE mark. This product meets the requirements for CE-IVD device under the EU Regulation for In Vitro Diagnostic Medical Devices (2017/746) |
| REF | Reference number |
|  | Manufacturer |
|  | Caution |
|  | Temperature limit |
|  | Expiration date |
| LOT | Lot number |

# 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 | 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 resuspen-sion 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 Dx 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 Dx vs sample volume was too high. Try adding less CleanNGS Dx during the size selection process to obtain larger DNA fragments (bp). |
| | Selected DNA fragments are too large (bp). | The ratio of CleanNGS Dx vs sample volume was too low. Try adding more CleanNGS Dx 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 Dx (50 mL) | CNGSDx-0050 |
| CleanNGS Dx (500 mL) | CNGSDx-0500 |

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

# Document Revision History

| IFU Version | Date of revision | Revised Chapter | Explanation of revision |
|---|---|---|---|
| 1 | 11/SEP/2024 | N/A | N/A |

# Notes

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