Clean Viral DNA/RNA Kit Special

USER MANUAL

Manual revision v5.00

Catalog Numbers:
CV-DR2304-SP: 2304 preps

Batch No: See package

Shipping: Room temperature

Storage and stability: CleanNA Particles VDR should be stored at 4°C upon receipt and Carrier RNA VDR at -20°C. Store all other components at room temperature. See page 3 for more storage information.

Intended use: Clean Viral 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 Clean Viral 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 Clean Viral DNA/RNA Kit Special allows for the viral RNA extraction from viruses, such as Coronavirus (SARS-CoV-2 / COVID-19), and total nucleic acid from nasopharyngeal SWABS, nasopharyngeal aspirates and bronchoalveolar lavage samples (BAL) samples in both Universal Transport Medium and Viral Transport Medium.

Our Clean Viral DNA & RNA Special Kit combines our propriety buffer system with the convenience of our CleanNA Particles to minimize the binding of PCR inhibiting compounds, present within the samples, onto our magnetic particles. Purified RNA is suitable for PCR, qPCR, RT-qPCR and other applications.

Using our specially formulated lysis buffer, samples are lysed and the nucleic acid is bound to our magnetic particles while DNases and RNases are efficiently deactivated. Nucleic Acid is isolated from the lysates in one step by binding to the CleanNA Particles' surfaces. The CleanNA magnetic particles are separated from the lysates using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants (e.g. proteins and cellular debris), the purified RNA is eluted from the CleanNA particles using molecular biology grade water or a low ionic strength buffer for use in downstream applications.

The isolation protocol is fully scalable and, due to the use of our magnetic bead purification technology, can easily be used both manually as automated on liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™). For automation of Viral RNA isolation on the CleanXtract 96, please use CleanNA's Viral RNA Swab kit.

The isolated Viral RNA is ready for use in downstream applications such as Next Generation Sequencing (NGS) and (q)RT-PCR.

Kit Contents and Materials

Kit Contents:

Product	CV-DR2304-SP	Storage
Preps	24 x 96	n/a
VDR Lysis Buffer	640 mL	15-25°C
Carrier RNA VDR	3 mg	-20°C
CleanNA Particles VDR	2x 26 mL	2-8°C
VDR Wash Buffer	500 mL	15-25°C
Molecular Biology Grade Water	250 mL	15-25°C

Note: Check the VDR Lysis Buffer for precipitates as precipitates may have formed during shipment or storage in cool ambient conditions. Precipitants can be dissolved by warming the VDR Lysis Buffer to 37°C and shaking gently.

Materials and Reagents to be supplied by User for clearance via centrifugation:

- 80% Ethanol, freshly prepared
- 100% Isopropanol
- Magnetic separation device, recommended Clean Magnet Plate 96-Well RN50 (Part# CMAG-RN50)
- 96-well microplates (Recommended ABgene® 1.2 mL storage plates, Cat# AB-1127) Working RNase Free

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.

Preparation of Reagents

Carrier RNA VDR

Dissolve the Carrier RNA VDR by adding molecular biology grade water to the tube containing lyophilized Carrier RNA VDR. Ensure to dissolve the Carrier RNA VDR thoroughly.

You may divide the carrier RNA into conveniently sized aliquots, ensuring the Carrier RNA VDR is not freeze-thawed more than 3 times. Store the Carrier RNA VDR solution at -20°C.

Kit	Molecular Biology Grade Water to be Added
CV-DR2304-SP	3 mL

VDR Wash Buffer

Dilute VDR Wash Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
CV-DR2304-SP	500 mL

Extraction of Viral DNA & RNA from NP Aspirates, SWABS and BAL samples

This protocol is designed for the extraction of viral DNA & RNA from nasopharyngeal aspirates, nasopharyngeal SWABS and bronchoalveolar lavage samples (BAL) in Universal Transport Medium (UTM) or Viral Transport Medium (VTM).

Before Starting:

- Prepare all reagents according the instructions on page 4.
- Sample material should be considered infectious, ensure to implement the required microbiology safety precautions.

Protocol:

1. Vortex the tubes containing the SWAB for 1 minute at maximum speed.
2. Transfer 200 µL of the sample into each well of a 96-well microplate.
3. Freshly prepare the following lysis master mix per sample.

Buffer	Volume/sample	Volume / 96 samples
VDR Lysis Buffer	240 µL	26.6 mL
Carrier RNA VDR	1 µL	105 µL

4. Transfer 240 µL lysis master mix to each well containing the supernatant from step 2. Vortex or pipet up and down 20 times to mix.
5. Add 280 µL Isopropanol and 20 µL CleanNA Particles VDR to each well. Mix by shaking for 10 minutes, or by pipetting up and down 20 times and then incubating for 10 minutes.
6. Place the plate on a magnetic separation device to separate the CleanNA Particles VDR. Incubate for 10-15 minutes until the CleanNA Particles VDR are completely cleared from solution.
7. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles VDR.
8. Remove the plate from the magnetic separation device.
9. Add 350 µL VDR Wash Buffer to each well.

Note: VDR Wash Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

10. Resuspend the CleanNA Particles VDR by vortexing for 3 minutes.

Note: Complete resuspension of the CleanNA Particles is required for adequate washing.

11. Place the plate on the magnetic separation device to separate the CleanNA Particles VDR. Incubate at room temperature until the CleanNA Particles VDR are completely cleared from solution.
12. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles VDR.
13. Remove the plate from the magnetic separation device.
14. Add 350 µL 80% ethanol to each well.

15. Resuspend the CleanNA Particles VDR by vortexing for 3 minutes.
16. Place the plate on the magnetic separation device to separate the CleanNA Particles VDR. Incubate at room temperature until the CleanNA Particles VDR are completely cleared from solution.
17. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles VDR.
18. Repeat Steps 13-17 twice for a total of 3 wash steps using 80% ethanol.
19. Leave the plate on the magnetic separation device for 15 minutes to air dry the CleanNA Particles VDR. Remove any residual liquid with a pipettor.
20. Remove the plate from the magnetic separation device.
21. Add 50-100 µL molecular biology grade water to each well.

Note: The required elution volume depends on plastic ware and magnetic separation device used. The CleanNA Particles VDR must be completely covered by the molecular biology grade water.

22. Resuspend the CleanNA Particles VDR by vortexing for 2 minutes.
23. Incubate at room temperature for 10 minutes.
24. Place the plate on the magnetic separation device to separate the CleanNA Particles VDR. Incubate at room temperature until the CleanNA Particles VDR are completely cleared from solution.
25. Transfer the cleared supernatant containing purified DNA/RNA to a clean plate.
26. Use the purified NA immediately of store at -70°C. Create appropriate aliquots to avoid repeated freeze/thawing.

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	RNA Degraded during storage.	Immediately process sample after collection or removal from storage.
	Incomplete Resuspension of Magnetic Particles.	Thoroughly resuspend CleanNA Particles VDR before use.
	80% ethanol not prepared correctly.	Prepare 80% ethanol with the correct amount of ethanol.
Problems in downstream applications	Insufficient RNA was used.	Quantify the purified DNA/RNA accurately and use sufficient DNA/ RNA.
		RNA in the sample already degraded, do not freeze and thaw the sample more than once or storage at room temperature.
	Ethanol carry-over.	Dry the CleanNA Particles VDR completely before adding elution buffer.
Carryover of Magnetic Particles	CleanNA Particles VDR would not fully separate on last step.	Place the eluted samples on a magnetic separation device for an additional 5 minutes or centrifuge at >4,000 x g for 5 minutes.

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Viral DNA & RNA Kit Special (24 x 96)	CV-DR2304-SP

Product	Part Number
Clean Magnet Plate 96-Well	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
5.00	October 2021	Total revision.	Language and layout modifications.
			Protocol clarifications
4.00	December 2020	Kit contents and Extraction procedure.	Updated volume of CleanNA Particles VDR.
3.00	September 2020	Total revision.	New lay-out.
			Important general user information added at page 1 (before contents).

Notes

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