Clean Viral RNA SWAB Kit

USER MANUAL

Manual revision v2.00

Catalog Numbers:
CV-R0384: 384 preps
CV-R2304: 2304 preps

Batch No: See package

Shipping: Room temperature

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

Intended use: Clean Viral RNA Swab Kit 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.

Quality Control: Each lot 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 RNA Swab Kit allows for the RNA extraction from viruses, such as Coronavirus (SARS-CoV-2 / COVID-19), from tracheal swabs such as nasopharyngeal and oropharyngeal swab samples in Universal Transport Media and Viral Transport Media.

Our Clean Viral RNA Swab Kit combines our propriety buffer system with the convenience of our CleanNA Particles VR 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 released into the lysate solution while RNases are deactivated. Viral RNA 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 nuclease free water or a low ionic strength buffer for use in downstream applications.

The protocol is scalable due to the use of our magnetic bead purification technology and can, besides manual usage, easily be automated on liquid handling workstations (e.g. CleanXTract 96, Dynamic Devices LYNX™, Hamilton STAR™). This user manual describes the use of the Clean Viral RNA Swab kit on the CleanXtract 96.

Kit Contents and Materials

Kit Contents:

Product	CV-R0384	CV-R2304	Storage
Preps	4 x 96	24 x 96	n/a
VR Lysis Buffer	110 mL	640 mL	15-25°C
Carrier RNA VR	1 mg	3 mg	-20°C
CleanNA Particles VR	2.2 mL	13 mL	2-8°C
VR Wash Buffer	100 mL	500 mL	15-25°C
Nuclease Free Water	60 mL	250 mL	15-25°C

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

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 96 Deep Well plates, 2.2 mL, V-Bottom; Cat# CXT-P096 or ABgene® 1.2 mL storage plates, Cat# AB-1127)

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 VR

Dissolve the Carrier RNA VR by adding nuclease free water to the tube containing lyophilized Carrier RNA VR. Ensure to dissolve the Carrier RNA VR thoroughly.

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

Kit	Nuclease Free Water to be Added
CV-R0384	1 mL
CV-R2304	3 mL

CleanNA Particles VR

Vortex the CleanNA Particles VR to completely resuspend the CleanNA Particles VR prior to usage.

VR Wash Buffer

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

Kit	100% Isopropanol to be Added
CV-R0384	100 mL
CV-R2304	500 mL

Extraction of Viral RNA from tracheal SWAB samples

This protocol is designed for the extraction of viral RNA from tracheal swabs such as nasopharyngeal swab and oropharyngeal swab samples 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. Make sure to implement the required microbiology personal safety precautions by following applicable guidelines.

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
VR Lysis Buffer	240 µL	26 mL
Carrier RNA VR	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 5 µL CleanNA Particles VR 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 VR. Incubate for 10-15 minutes until the CleanNA Particles VR are completely cleared from solution.
7. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles VR.
8. Remove the plate from the magnetic separation device.
9. Add 350 µL VR Wash Buffer to each well.

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

10. Resuspend the CleanNA Particles VR 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 VR. Incubate at room temperature until the CleanNA Particles VR are completely cleared from solution.
12. Aspirate and discard the supernatant. Do not disturb the CleanNA Particles VR.
13. Remove the plate from the magnetic separation device.
14. Add 350 µL 80% ethanol to each well.
15. Resuspend the CleanNA Particles VR by vortexing for 3 minutes.
16. Place the plate on the magnetic separation device to separate the CleanNA Particles VR. Incubate at room temperature until the CleanNA Particles VR are completely cleared from solution.

17. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles VR.
18. Repeat Steps 13-17 once for a total of 2 wash steps using 80% ethanol.
19. Leave the plate on the magnetic separation device for 15 minutes to air dry the CleanNA Particles VR. Remove any residual liquid with a pipettor.
20. Remove the plate from the magnetic separation device.
21. Add 50-100 µL nuclease free water to each well.

Note: The required elution volume depends on plastic ware and magnetic separation device used. The CleanNA Particles VR must be completely covered by the nuclease free water.

22. Resuspend the CleanNA Particles VR by shaking 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 VR. Incubate at room temperature until the CleanNA Particles VR are completely cleared from solution.
25. Transfer the cleared supernatant containing purified RNA to a clean plate.
26. Use the purified NA immediately or store at -80°C. Create appropriate aliquots to avoid repeated freezing/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 VR 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 RNA accurately and use sufficient RNA. RNA in the sample already degraded, do not freeze and thaw the sample more than once or store at room temperature for too long.
	Ethanol carry-over.	Dry the CleanNA Particles VR completely before adding elution buffer.
Carryover of Magnetic Particles	CleanNA Particles VR 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	Preps	Part Number
Clean Viral RNA Swab Kit (4 x 96)	384	CV-R0384
Clean Viral RNA Swab Kit (24 x 96)	2304	CV-R2304

Product	Pack Size	Part Number
96 Deep Well Plate (2.2 mL; V-bottom)	50 pcs/box	CXT-P096
Clean Magnet Plate 96-Well	1 Plate	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
2.00	October 2021	Introduction and Principle.	Text revisions and adapted list of liquid handlers.
		Total document.	Typo's corrected, text rephrased.
		Kit contents and materials.	Added Cat# CXT-P096 as recommended 96 well plate.
			Updated volume of CleanNA Particles VR to 2.2 mL for the CV-R0384 kit.
1.00	July 2021	Total document.	New document.

Notes

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