Clean Viral DNA & RNA Kit Special

Catalog Nos. CV-DR2304-SP
Manual revision v1.00

Contents

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Introduction and Principle

The Clean Viral DNA/RNA Kit Special allows for the RNA extraction from Coronavirus (SARS-CoV-2 / COVID-19), extraction total nucleic acid from nasopharyngeal SWABS, nasopharyngeal aspirates and bronchoalveolar lavage samples (BAL) samples in Universal Viral Transport Media.

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 DNA is suitable for PCR, restriction digestion, and hybridization 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 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 by using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants (e.g. proteins and cellular debris), the purified DNA and RNA is eluted from the CleanNA particles for downstream applications using an nuclease-free water or a low ionic strength buffer.

The kits protocol is fully scalable and due to the use of our magnetic bead purification technology and can, besides manual usage, easily be automated on liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™, Biomek™ i5, Biomek™ i7, Thermo KingFisher™ Flex, Applied Biosystems® MagMAX™, Qiagen BioSprint, and other liquid handling instruments).

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Kit Contents and Materials

Kit Contents:

Product	CV-DR2304-SP	Storage
Preps	24 x 96	
VDR Lysis Buffer	640 mL	15-25^{0}C
Carrier RNA VDR	3 mg	-20^{0}C
CleanNA Particles VDR	26 mL	2-8^{0}C
VDR Wash Buffer	500 mL	15-25^{0}C
Nuclease-free Water	250 mL	15-25^{0}C

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 gently shaking.

Materials and Reagents to be supplied by User for Tissue and Serum & Stool protocol:

- 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)

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Preparation of Reagents

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

Kit	Nuclease free water to be Added
CV-DR2304-SP	3 mL

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.

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

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

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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).

Before Starting:

- Prepare all reagents according to the “Preparation of Reagents” section on Page 4.

Protocol for nasopharyngeal aspirates, nasopharyngeal SWABS and bronchoalveolar lavage samples in Transport Medium (UTM) or Viral Transport Medium (VTM):

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.
5. Add 280 µL Isopropanol and 10 µL CleanNA Particles VDR to each well. Mix by shaking for 5 minutes, or by pipetting up and down 15 times and then incubating for 5 minutes.
6. Place the plate on a magnetic separation device to separate the CleanNA Particles VDR. Incubate for 10-15 minutes.
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 shaking for 1 minute.

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 shaking for 1 minute.

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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 for a second 80% ethanol wash step.

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 Nuclease-free Water to each well.

    Note: The required elution volume depends on plastic ware and magnetic separation device used. The CleanNA Particles VDR must be able to completely covered by the Nuclease-free Water.

22. Resuspend the CleanNA Particles VDR 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 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. Store at -70°C.

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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 store at room temperature for too long
	Ethanol carry-over	Dry the CleanNA Particles VDR completely before adding elution buffer.
Carryover of Magnetic Beads	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.
Abnormal BioAnalyzer data	BioAnalyzer shows multiple sharp peaks during the analysis	Ensure to remove all traces of the cleared supernatant after each wash step
	BioAnalyzer shows base line climbing towards the end	Check the BioAnalyzer chip for air bubbles. Load samples onto a new freshly prepared chip
	BioAnalyzer shows high blob at the beginning of the trace	Ensure the purified sample does not contain traces of CleanNA Particles CC

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Ordering Information

Contact your local distributor to order.

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

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

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