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DATA SHEET

Version: 03
Revision date: 19/04/2023

1. Identification

Product name **CVX Viral RNA Extraction Kit, CE-IVD**

Cat. No **AN0105 (100 reactions)**
Cat. No **AN0105-XL (500 reactions)**

2. Description

CVX Viral RNA MiniSpin Kit is designed for the rapid simultaneous purification of viral RNA from cell – free samples such as serum, plasma, urine, cell free body fluids, cell culture supernatants and rinse liquid from swabs samples.
The viral RNA molecules bind to the silica-based media and impurities such as proteins and nucleases are removed by thorough washing with Wash Buffer. The RNA is then eluted in sterile, RNase free water. The isolated viral RNA is ready to use and should be stored at - 70˚C.
The procedure can be used for isolation of viral RNA from a broad range of viruses. However, performance cannot be guaranteed for every virus species and must be validated by the costumer. The amount of purified viral RNA depends on the sample type, the virus titer, sample source, transport, storage, and age. The Kit also includes carrier RNA that improves binding and recovery of low-concentrated viral RNA.

3. Composition

Item	Quantity	
	AN0105 (100 rxn)	AN0105-XL (500 rxn)
Minispin-columns	100	500
Collection tubes (2 ml)	200	1000
Carrier RNA (lyophil)	1 vial	1 vial
BLQ Buffer	60 ml	300 ml
WB1 Buffer*	33 ml	165 ml
WB2 Buffer*	20 ml	100 ml
RNase-free Water	10 ml	50 ml

4. Quality Control

The quality of **CVX Viral RNA MiniSpin Kit** is tested on a lot-to-lot basis by isolating viral RNA from a 140 µl serum sample.

5. Storage specifications

Store the vial of lyophilized Carrier RNA at –20 ˚C and all other components at room temperature (18 to 25 ˚C). If any kit reagent forms a precipitate, warm at 55–65 ˚C until the precipitate dissolves, and allow cooling to room temperature before use.
*Add the volume ethanol (96%-100%) specified [Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

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6. Applications

The purified viral RNA is suitable for use in RT-PCR and qRT-PCR and can be used for:

- Viral load monitoring
- Viral detection
- Viral genotyping

7. Product use limitation

This product is developed, designed and tested for both research purposes and in vitro diagnosis (IVD). The product has not been tested for use in drug development, nor is suitable for administration to humans or animals. Please refer to **www.canvaxbiotech.com** for Material Safety Data Sheet (MSDS) of the product.

8. Equipment needed and compatibility

EQUIPMENT NEEDED FOR MANUAL RNA ISOLATION:

1. Biological Safety Cabinet suitable for work with potentially infectious samples. Please follow local guidelines for working with potentially infectious material in particular if material is derived from a human or animal sample.
2. **Micropipettes** suitable for pipetting 50 µL, 140 µL, 500-700 µL.
3. **Vortex.**
4. **Microcentrifuge** suitable for an average RCF (Relative Centrifugal Force) of at least 10.000g. The relationship between Revolutions per Minute (RPM) and RCF is as follows

   RCF=(1,118 x 10-5)x radius(cm) x (RPM)2

   wherein radius is the radius (in centimeters) of the rotor from the center to the center of the tube inserted on it. For example, if a microcentrifuge has a rotor with a minimum radius of 5,5cm, then 13.000RPM are equivalent to about 10.400RCF. As fixed-angle rotors have an inclination there is a minimum radius to the top of the microtube, an average radius to the middle of the microtube and a maximum radius to the bottom of the microtube. Thus, as a rule of thumb, for the following minimum radius of a rotor the RPM needed to centrifuge at 10.000g are shown in the next table

Radius (cm)	RCF (g)	RPM
4,5	10.000	14.100
5	10.000	13.400
5,5	10.000	12.800
6	10.000	12.200
6,5	10.000	11.800
7	10.000	11.300

   The use of a microcentrifuge and micropipettes for RNA isolation is often called "manual isolation" protocol as every step is conducted by a skilled technician (e.g. a scientist or a dedicated staff on a clinical lab).

5. -20°C freezer for storage of RNA carrier and Ultra-Low Temperature Freezer (for storage of isolated samples at - 80°C).

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EQUIPMENT NEEDED FOR AUTOMATED RNA ISOLATION:

1. **Biological Safety Cabinet** suitable for work with potentially infectious samples. Please follow local guidelines for working with potentially infectious material in particular if material is derived from a human or animal sample.
2. **Micropipettes** suitable for pipetting 50 µL, 140 µL, 500-700 µL.
3. **Vortex.**
4. **Robotic workstation** for nucleic acid purification using silica-based individual minicolumns, e.g. **QIAcube.**

This kit is **NOT compatible** with magnetic-based robotic workstations nor with microplate-based robotic workstations.

9. Material needed but NOT supplied

MATERIAL NEEDED FOR MANUAL ISOLATION:

1. Material needed to collect the biological sample.
2. **Personal Protective Equipment (PPE):** Please follow local guidelines for working with potentially infectious material in particular if material is derived from a human or animal sample.
3. **Plastic Disposable Tips** sterile with filter and RNAse-free for micropipettes or for robotic workstation.
4. **Sterile 1,5mL microcentrifuge tubes** (RNAse-free).
5. **Ethanol 96%-100% (for example Ethanol BioUltra for molecular biology, from Sigma-Aldrich 51976)**

BEFORE STARTING

1. Please read carefully the whole protocol and be sure to fully understand it before starting.
2. Resuspend the supplied lyophilized vial of **Carrier RNA using 600 µL** (for Cat. No. AN0105, 100 reactions) or **3.000 µL (for Cat. No. AN0105XL, 500 reactions)** of supplied RNAse-free Water and mix thoroughly. Carefully design the expected number of isolations you are going to do per week and make aliquots of resuspended Carrier RNA accordingly and store them at -80°C for up to 6 months. **Carrier RNA** enhances binding of viral RNA to the silica membrane and reduces the risk of viral RNA degradation but **Carrier RNA** has a limited shelf-life of 4 weeks in **BLQ Buffer** when stored at 4°C. In addition, more than 3 freeze-thaw cycles of **Carrier RNA** **MUST** be avoided. Use the following table as a guideline:

RNA preps	BQL Buffer	Carrier RNA
12	7,2 ml	72 µL
24	14,4 ml	144 µL
48	28,8 ml	288 µL
96	57 ml	570 µL
100	60 ml	600 µL
192	115 ml	1,15 ml
384	230 ml	2,30 ml
480	288 ml	2,88 ml
500	300 ml	3 ml

Thus, if very few RNA isolations are going to be done per week, it is advisable to make several **Carrier RNA** aliquots to avoid both freeze-thawing and storing **BLQ Buffer** containing **Carrier RNA** for a long time. On average about 600 µL of **BLQ Buffer** is prepared for each RNA isolation. For example, if **Cat. No. AN0105XL** is purchased and the expected number of RNA isolations per week is low (e.g. <24 RNA isolations/week) then make up to 10 aliquots of **300 µL** each of resuspended Carrier RNA and store them at -80°C.

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3. Prepare **BLQ Buffer** containing **Carrier RNA** following above guidelines, mark the label of the bottle to indicate that **Carrier RNA** has been added and store unused **BLQ Buffer** at 4°C for up to 4 weeks. Record the date of Carrier RNA addition to **BLQ Buffer.**

4. Add the specified volume of Ethanol (96%-100%) for Molecular Biology to **WB1 Buffer** and **WB2 Buffer** (see volume of ethanol on the bottle label) and mark the bottle to indicate that this step has been done.

5. Although for isolation of viral RNA, Proteinase K treatment is usually not required, it is recommended for isolation from viscous samples (e.g., sputum samples). Add 25µL Proteinase K (20 mg/ml stock solution), to the lysis mixture and mix by vortexing vigorously for 5 seconds. Incubate for 5 min at 70°C. Please note that Proteinase K is **NOT** included in the kit. Alternatively, if the sample is very viscous and there is no further reaction of isolated RNA, then a more diluted sample may be used for a new RNA isolation, for example a 1/2 to 1/10 of starting sample may be used.

DETAIL PROTOCOL

1. Transfer **560 µL** of **BLQ Buffer** (**containing Carrier RNA**) into a 1,5 mL microcentrifuge tube (not provided).
• ***Carrier RNA*** enhances binding of viral RNA to the silica membrane and reduces the risk of viral RNA degradation.

2. Add **140 µL** of sample (plasma, serum, urine, body fluids or cell cultured supernatant) and mix by vortexing for 5 seconds.
• ***Nasopharyngeal swab (NP) /oropharyngeal swab (OP):*** If the swab is delivered in a transport media suitable for nucleic acid virus stabilization, transfer 140 µL directly into microcentrifuge tube. If you get a swab without transport media, place the swab with into microcentrifuge tube containing PBS and incubate for 15 minutes at room temperature. Afterwards shake the swab vigorously, squeeze it against the wall of tube and remove the swab. Use a 140 µL aliquot of the liquid for viral RNA extraction.
• ***Internal Extraction Control:*** When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer (BLQ-Buffer). This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. DO NOT add the internal control RNA directly to the biological sample as this will lead to degradation and a loss in signal strength.

3. Incubate the mix at room temperature for 10 minutes.

4. Add **560 µL** of ethanol (96-100%) to the sample, and mix by vortexing for 5 seconds.

5. Place **Minispin-column** in a 2 ml Collection tube and transfer 700 µL of the lysed sample. Centrifuge at least at 10.000xg for 1 minute. Normally it is best to centrifuge at maximal speed Discard the flow-through.
• For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and no viscous sample before loading onto the **Minispin- Column.**

6. Repeat Step 5 until all the sample has been transferred to the Minispin-column.

7. Place the **Minispin-column** in a new Collection tube and add 500 µL of **WB1 Buffer**. Centrifuge at least at 10.000xg (or maximal speed) for 1 minute. Discard the flow-through.

8. Place the **Minispin-column** in the same Collection tube and add 500 µL of **WB2 Buffer**. Centrifuge at 10.000xg (or maximal speed) for 1 minute. Discard the flow-through.

9. Place the **Minispin-column** in the same Collection tube and add 500 µL of **WB2 Buffer.** Centrifuge at 10.000xg (or maximal speed) for 1 minute. Discard the flow-through.

10. Centrifuge at full speed for an additional 3 min to dry the **Minispin-column**. Rotate the column 180° and repeat centrifugation at full speed for an additional 3 min to dry the **Minispin column**.
• This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

11. Place the **Minispin-column** into a new, labelled 1.5 microcentrifuge tube (not provided) and pipet 50 µL **RNasefree Water** directly into the membrane. Close the cap and incubate for 2 minutes at room temperature.

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12. Centrifuge at full speed for 1 minute to elute. The eluate contains viral RNA. After extraction place the Elution
Tube on ice. For long time storage place the nucleic acids at –80˚C.
• Final eluates contain viral RNA and Carrier RNA; therefore, it is not possible to quantify the nucleic acids isolated with the kit by photometric or fluorometric methods.

PROTOCOL VALIDATION FOR COVID-19

CVX Viral RNA MiniSpin Kit has been validated for RNA isolation from SARS-CoV-19 clinical samples. 60 positive samples and 20 negative samples were collected from nasopharyngeal exudates and RNA isolation was made in parallel using **CVX Viral RNA Minispin Kit** and a comparator kit. After RNA isolation qPCR was done using the protocol described by Corman VM et al, 2020, also known as "Charité protocol". An internal mRNA co-isolated along with viral RNA was used as a positive control.

GENERAL PROCEDURE