CVX™ Viral RNA Extraction Kit

User Manual, Revision V.01

REF **AN0105; AN0105-XL**

MANUFACTURED BY
Canvax Biotech SL, Córdoba, Spain

FOR SINGLE USE ONLY
This kit is made for single use only!

10°C 30°C
STORAGE CONDITIONS
Store at Room Temperature or shown conditions respectively.

USED BY
Expiry date.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CE IVD The kit is an In-Vitro Diagnostic medical product

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1. Description

CVX™ Viral RNA MiniSpin Kit is designed for the rapid 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 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 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 kit also includes carrier RNA that improves binding and recovery of low-concentrated Viral RNA.

1.1. Intended Use

This product is developed, designed and tested for both Research purposes (RUO) 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.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

1.2. Applications

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

- Viral load monitoring.
- Viral detection.
- Viral genotyping.

1.2.1. COVID-19 RNA Extraction validation

CVX™ Viral RNA MiniSpin Kit has been validated for RNA isolation from SARS-CoV-19 clinical samples. 59 positive samples and 20 negative samples were collected from nasopharyngeal exudates and RNA isolation was performed in parallel using **CVX™ Viral RNA Minispin Kit** and a comparator kit at Centro Nacional de Microbiología (CNM, ISCIII, Madrid). After RNA isolation qPCR was done using the protocol described by Corman VM et al, 2020, also known as the "Charité protocol".

An internal mRNA co-isolated along with Viral RNA was used as a positive control. If in a sample there is no amplification of the internal control by qPCR, then this reaction is considered inhibited.

The Sensitivity of the kit was 98,3% and the Specificity was 100%. The only false negative by CVX™ Viral RNA Minispin Kit was a sample where the Ct value measured by the Reference RNA isolation kit was 36,8.

KIT		Reference RNA isolation Kit (CNM, ISCIII)		
		(+)	(-)	Total
CVX™ Viral RNA MiniSpin Kit	(+)	58	0	58
	(-)	1	20	21
	Total	59	20	79

1.3. Quality Control and Product Warranty

All products sold by the Canvax Biotech are subjected to extensive Quality Control procedures and are warranted to perform as described when used correctly. The performance of all components of the **CVX™ Viral RNA Extraction Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

Should any Product fail to perform the applications as described in the manual, Canvax Biotech will check the lot and if Canvax Biotech investigates a problem in the lot, Canvax Biotech will replace the Product free of charge.

Canvax Biotech reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

2. Kit Components

2.1. Kit Contents

	Catalog Number	
ITEM	AN0105	AN0105-XL
MiniSpin Columns	100	500
Collection Tubes (2 mL)	200	1,000
Carrier RNA	1 vial	1 vial
BLQ Buffer [a]	60 mL	300 mL
WB1 Buffer [a, b]	33 mL	165 mL
WB2 Buffer [b]	20 mL	100 mL
RNAse Free Water	10 mL	50 mL

[a] *Contains chaotropic salts. See safety information on page 5.*

[b] *Add the specified volume Ethanol (96%-100%) [Not included] 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.*

2.2. Shipping and Storage

The kit is shipped at Room Temperature. However, in some specific cases, Carrier RNA may be shipped separately in Dry Ice.

Store the vial of lyophilized Carrier RNA at –20 ˚C and all other components at Room Temperature (18 to 25 ˚C); columns should be dry stored. If any kit reagent forms a precipitate, warm to 55–65 ˚C until the precipitate dissolves, and allow cooling to Room Temperature before use.

2.3. Safety Information

Please consult the Safety Data Sheet (SDS) associate to this product. It is available online in PDF format.

The kit contains Guanidine Salts Buffers that may produce hazardous gases when combined with bleach (sodium hypochlorite) and/or strong acids.

If these Buffers are spilt, clean the surface with Water and a suitable laboratory detergent. If is necessary surface desinfection then, use 1% (v/v) Sodium Hypochlorite when the area is cleaned.

2.4. Equipments and Materials supplied by the user

2.4.1. Equipment needed for manual RNA isolation

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). The equipment needed are:

- **- 20 ˚C Freezer** for storage of carrier RNA and Ultra-Low Temperature Freezer (for storage of isolated samples at -80 ˚C).
- **Biological Safety Cabinet** suitable for work with potentially infectious samples. Please follow local guidelines for working with potentially infectious material in particular if the material is derived from a human or animal sample.
- **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
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$$RCF = (1{,}118 \times 10^{-5}) \times \text{radius (cm)} \times (RPM)^2$$

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 5.5 cm, then 13,000 RPM are equivalent to about 10.400 RCF. 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

- **Micropipettes** suitable for pipetting 50 µL, 140 µL, 500-700 µL.
- **Vortex**.

2.4.2. Equipments needed for automated RNA isolation

This kit is NOT compatible with magnetic-based robotic workstations nor with Microplate-based robotic workstations. It could be used in QIACube® workstations.

The equipments needed are:

- **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.
- **Micropipettes** suitable for pipetting 50 µL, 140 µL, 500-700 µL.
- **Vortex**.
- **Robotic Workstation** for nucleic acid purification using silica-based individual MiniSpin Columns, e.g. QIAcube®.

2.4.3. Materials not included

- Material needed to collect the biological sample.
- Personal Protective Equipment (PPE): Please follow local guidelines for working with potentially infectious material in particular if the material is derived from a human or animal sample.
- Plastic Disposable Tips sterile with filter and RNAse-free for micropipettes or for robotic workstation.
- Sterile 1.5 mL Microcentrifuge tubes (RNAse-free).
- Ethanol for Molecular Biology 96%-100% (for example Ethanol BioUltra for Molecular Biology, from Sigma-Aldrich 51976).

3. Procedure

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

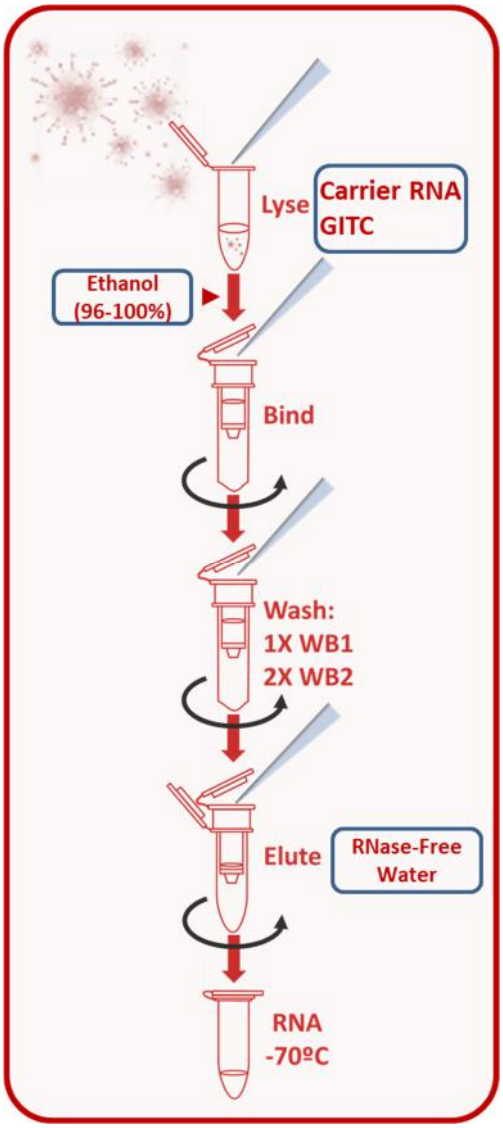
Thus, if very few RNA isolations are going to be done per week, it is advisable to make several **Carrier RNA** aliquots to avoid both freeze-thawing and storing **BLQ Buffer** containing **Carrier RNA** for a long time. On average about **600 µL** of **BLQ Buffer** is prepared for each RNA isolation. For example, if **Cat. No. AN0105-XL** 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.

3. Prepare **BLQ Buffer** containing **Carrier RNA** following above guidelines, mark the bottle label 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.

3.2. Workflow



3.3. Procedure

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 a 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 **Mini-spin-column.** Rotate the column 180° and repeat centrifugation at full speed for an additional 3 min to dry the **Mini-spin column.**

    This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

11. Place the **MiniSpin Column** into a new, labelled 1.5 mL Microcentrifuge tube (not provided) and pipet 50 µL **RNase-free Water** directly into the membrane. Close the cap and incubate for 2 minutes at Room Temperature.

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.

4. Troubleshooting

Low yield	1. RNA Carrier: • RNA Carrier not added. • RNA Carrier degraded: This reagent should be storage at -20 ˚C. 2. Viral nucleic acids degraded: • Samples must be storage appropiately. • Repeated freezing and thawing of the simples should be avoided. • Keep in mind RNA work cares. 3. Insufficient Lysis: • Increase lysis time. • Reduce amount of starting material. • Overloading of Spin-Column reduces yield! 4. Ethanol: • Ethanol was not added to the indicated Buffers. • Use 96-100% Ethanol, not denaturated Ethanol. 5. Buffers WB1 and WB2 were used in incorrect order.
RNA degradation	1. Ensure the samples are adequately processed. 2. Keep in mind RNA work cares.
Low detection	1. Ethanol carry-over. Traces of Ethanol from the Wash Buffer2 can inhibit downstream enzymatic reactions: • Prolong centrifugation steps in order to remove Buffer WB2 completely.