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DATA SHEET

Version: 02
Revision date: 19/06/2023

1. Identification

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

Cat. No **AN0501 (100 reactions)**
Cat. No **AN0501-XL (500 reactions)**

2. Description

CVX-Mag Viral RNA Extraction Kit designed for rapid manual and automated 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. Extraction is based on paramagnetic beads technology.
The extraction process includes an initial lysis step with the appropriate Buffer to ensure efficient lysis and nucleic acid release from the virus, Nucleic acid binding to the surface of the magnetic beads, washing and elution.
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.
No centrifugation is necessary in this magnetic nucleic acid purification process, which saves on equipment costs and increases portability of the technique.

3. Composition

Item	Quantity	
	AN0501	AN0501-XL
CVX Magnetic Beads[1]	2 mL	10 mL
BLQ-Buffer	20 ml	100 ml
Proteinase K (Lyophilizate)	30 mg	5 x 30 mg
PK Resusupension buffer	1.5 mL	5 x 1.5 mL
Carrier RNA (Lyophilized)	0.2 mg	1 mg
WB1 Buffer[2]	33 ml	165 ml
WB2 Buffer[2]	20 ml	100 ml
RNase-free Water	15 ml	60 ml

NOTES:

1 Do not freeze CVX Magnetic Beads.

2 Add the specified volume of Ethanol Molecular Biology grade (96%-100%) [Not included] to WB1 and WB2 Buffer (see volume of Ethanol on the bottle label) and mark the bottle to indicate that this step has been done. Do not use denatured ethanol, as it contains components not compatible with the protocol.

CE IVD The **CVX-Mag Viral RNA Extraction Kit** TESTED AND VALIDATED FOR RNA EXTRACTION OF SARS-CoV-2 (COVID-19).

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4. Storage

Store the Carrier RNA(Lyophilized) and Proteinase K (Lyophilized) at -20°C and all other components at room temperature (+15 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.

5. Applications

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

- Viral load monitoring
- Viral detection
- Viral genotyping

6. Quality Control

The quality of **CVX-Mag Viral RNA Extraction Kit** is tested on a lot-to-lot basis by isolating viral nucleic acid from a 200µl sample.

Before starting:

1. **Proteinase K:**
   When using the kit for the first time, add 1.5 mL PK Resuspension Buffer to the lyophilized Proteinase K. The Proteinase K solution can be stored for several days at 2–8 ˚C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 ˚C until needed. This product as supplied is stable at room temperature.

2. **For automatic purification:**
   Prepare CVX Magnetic Bead Suspension: A homogeneous distribution of the magnetic beads is essential for a high well-to-well consistency. Therefore, before distributing the beads, make sure that the beads are completely resuspended. Shake the storage bottle well or place it on a vortexer shortly. **Premixing magnetic beads with Isopropanol (99.7%, molecular biologic grade) [Not included in the kit] allows easier homogenous distribution of the beads to the individual wells of the separation plate.** During automation, a premix step before aspirating the **Magnetic Bead Suspension** from the reservoir is recommended to keep the beads resuspended.

   Prepare the following CVX Magnetic Bead Suspension for 96 samples: Immediately before use, add 38 mL Isopropanol to CVX Magnetic Beads then mix by vortexing to completely resuspend. Mark the bottle to indicate that the Isopropanol was added. Be sure and close the bottle tightly after each use to avoid Isopropanol evaporation.

RNA preps	CVX Magnetic Beads	Isopropanol (100%)
100	2 mL	38 mL
500	10 mL	190mL

3. Immediately before use, resuspend the supplied lyophilized vial of **Carrier RNA** to obtain a solution of 1 µg/µL using **200 µL** 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 magnetic beads 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 solution MUST be avoided.** Use the following table as a guideline:

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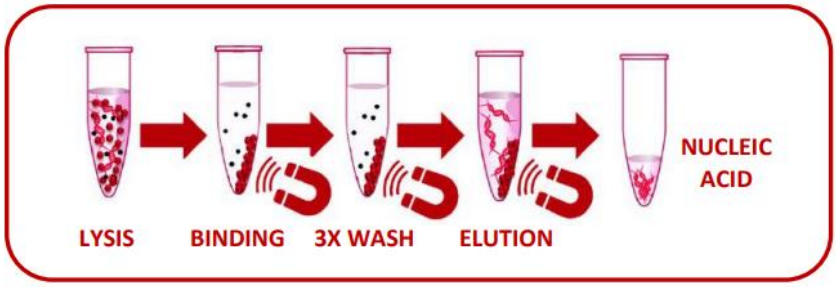
RNA preps	BLQ Buffer	Carrier RNA (1µg/µl)
25	5 mL	50 µL
50	10 mL	100 µL
100	20 mL	200 µL
500	100 mL	500 µL

Mark the bottle label to indicate that Carrier RNA has been added.

Protocol:

This protocol is intended for manual use of the kit. It can also be used as a guideline to set up an automated procedure on liquid handling instruments.



1. Add **200 µL of sample** (plasma, serum, urine, body fluids or cell cultured supernatant) into a microtube (or well plate). If you are processing < 200 µL sample, adjust final volume of the sample to 200 µL using PBS (phosphate buffered saline) or 0.9% NaCl.
   - *Nasopharyngeal swab (NP) /oropharyngeal swab (OP): If the swab is delivered in a transport media suitable for nucleic acid virus stabilization, transfer 200 µl directly into microtube (or well plate). If you get a swab without transport media, rinse swab with cooled PBS and use a 200 µl aliquot of the liquid for viral RNA extraction.*
2. Add **200 µL of BLQ Buffer** (containing **carrier RNA**) and briefly vortex to mix.
3. Add **15µL Proteinase K** (20 mg/ml stock solution), to the lysis mixture and mix by vortexing vigorously for 20 seconds.
   - ***Proteinase K treatment**: Although for isolation of viral RNA, Proteinase K treatment is usually not required, it is recommended for isolation from viscous samples (e.g., respiratory samples) and simultaneous extraction of viral RNA/DNA.*
4. Incubate the mix for 10 minutes at 70°C. Shake and mix occasionally.
5. Add **20 µL CVX Magnetic Beads** and 380 µL Isopropanol (99.7%) and mix gently by vortexing (or by repeated pipetting up and down) then incubate at room temperature for 5 minutes to ensure complete binding of the RNA (Occasionally shake and mix).
   - *Before distributing the beads, make sure that beads are completely resuspended. Shake storage vial well or vortex briefly.*
   - *Premixing magnetic beads with isopropanol allows easier homogenous distribution of the magnetic beads to the microtube (well plate).*

RNA preps	CVX Magnetic Beads	Isoprpanol (100%)
1	20 µL	380 µL
25	0.5 mL	9.5 mL
50	1 mL	19 mL
100	2 mL	38 mL
500	10 mL	190 mL

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6. Place on magnetic separator for 5 minutes (or until the magnetic beads clear from solution) and discard the supernatant using a pipette.
   - *Do not disturb the attracted beads while aspirating the supernatant. Angle the pipette tip away from the bead pellet to avoid contact.*
7. Remove the plate from the magnetic separator and add **500 µL of WB1 buffer**. Resuspend the beads by vortexing until the beads are resuspended completely (Alternatively you can resuspend the beads by repeated pipetting up and down or on a shaker for 5 min at 1000 RPM).
8. Place the samples in a magnetic separator and wait at least 2 minutes to collect the beads (or until solution clarifies). Remove the supernatants without disturbing the attracted bead pellet.
9. Remove the plate from the magnetic separator and add **500 µL of WB2 Buffer**. Resuspend the beads by vortexing until the beads are resuspended completely (Alternatively you can resuspend the beads by repeated pipetting up and down or on a shaker for 5 min at 1000 RPM).
10. Place the samples in a magnetic separator and wait at least 2 minutes to collect the beads (or until solution clarifies). Remove the supernatants without disturbing the attracted bead pellet.
11. Repeat steps 9-10 for a **second wash**.
    Use pipette with 200µl tip to effectively remove all WB2 Buffer.
12. Open the cap and dry at 30°C for 10 minutes until the ethanol volatilizes completely.
13. Add **50-100 µL Nuclease-free water** and mix gently by vortexing (or pipette up and down slowly 8-10 times) and incubate at room temperature at least 10 minutes during which shake and mix occasionally (or continuously on a shaker for 10 min at 1000 RPM).
    - *Note that the dry/flaky beads are difficult to resuspend. If the magnetic particle pellet attaches to the tube wall tightly, place the tubes in the bench top microfuge with the bead pellet oriented toward the center, and spin for 30 seconds to detach the magnetic particles into the elution buffer. If it is difficult to resuspend the magnetic particles, use P200 to pipette up-and-down several times to resuspend the particles. Be careful not to let the particle pellet stick inside the tip.*
14. Place on magnetic separator for 2 minutes (or until solution clarifies).
15. Transfer the supernatant (which contains the purified RNA) to a new clean microtube (Plate).
    - *For long time storage place the RNA at –80˚C C.*
    - *Magnetic Beads can be PCR inhibitory: If the transferred eluates contain magnetic Beads, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes (or centrifuge the microtube (plate) at top speed for 3 min to pellet residual magnetic particles and transfer the liquid phase with eluted RNA to a new microtube (plate) without disturbing the Magnetic particles).*

Procedure on integrated Robotic System:

Established automation workflows on various open access automation platforms including OPENTRONS, HAMILTON, KINGFISHER and other devices. Important: If automating this procedure on a liquid handler or a magnetic processor, please contact your Canvax Biotech representative for instrument-specific instructions.

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Protocol for the isolation of Viral RNA on KingFisher Flex:

1. Set up the plates:
   Use standard 96 deep well plates compatible with KingFisher™ Flex. Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table:
   Prepare the processing plates:

Plate Position	
2	Washing Plate 1: Add 500 µl WB1 Buffer to a 2.0 ml Deep Well Plate
3	Washing Plate 2: Add 500 µl WB2 Buffer to a 2.0 ml Deep Well Plate
4	Washing Plate 3: Add 500 µl WB2 Buffer to a 2.0 ml Deep Well Plate
5	Elution Plate: Add 70 µl RNAse Free Water to Elution Plate
6	Tip Comb Plate: Place a 96 Deep-well Tip Comb in a Standard Plate

   Prepare sample plate (200 µL sample input volume):

Plate #	
1	• **Sample Plate (Lysis/Binding):** Add **200 µl of BLQ Buffer (containing carrier RNA)** to each well of the Sample Plate and 200 µl sample. Mix by pipetting up and down until a homogeneous mixture is visible (typically 3 – 4 times). • Add **15 µL Proteinase** K to the lysis mixture and mix by • Incubate at 70°C for 10 min. • Add **400 µL CVX Magnetic Beads** suspension and Mix gently by repeated pipetting up and down. *Before distributing the beads, make sure that beads are completely resuspended. Shake storage botle well or vortex briefly.*

2. Settings of KingFisher FLEX and automated extraction run:

   Start the run:
   Select the program **MVP_Flex_200ul.bdz** on the instrument.
   Start the run. Load the prepared plates into the indicated position when prompted by the instrument.

   Collect RNA:
   After the run is complete, remove the elution plate from the instrument.