PATHKITS

Version: 7.0

MDS™ Viral RNA Extraction Kit

Product Code: MDS011

Kit Contents (250 Reactions)

MDS Viral RNA Extraction Kit	Quantity	Packaging
Spin columns	250	Zip Pouch
Collection Tubes (2 ml)	500	Zip Pouch
Centrifugal Tubes (1.5 ml)	250	Zip Pouch
Buffer 1	1 X 50 ml	1 x 50ml Bottle
Buffer 2	1 X 25 ml	1 x 50ml Bottle
Buffer 3	1 X 25 ml	1 x 50ml Bottle
Buffer 4	1 X 75 ml	2 x 50ml Bottle
Wash Buffer 1	1 X 150 ml	3 x 50ml Bottle
Wash Buffer 2	1 X 150 ml	3 x 50ml Bottle
Elution Buffer	1 X 25 ml	1 x 50ml Bottle

*Ethanol is not supplied with the kit
*Preheat Buffer 2 before starting the extraction (In order to dissolve the precipitates).
*Store all the buffers at room temperature.
*If your RT PCR KIT is supplied with an exogenous internal control (IC), then the EIC supplied with your RT PCR kit has to be added in step 3 as per chart below.
*If your RT PCR KIT is supplied with an endogenous internal control , then the IC supplied with your RT PCR kit need not be added in step 3 as per chart below.

RNA Extraction Protocol (Short Protocol)

Steps	Instructions	Centrifuge
Step 1	Take 100 µl of VTM in a micro-centrifuge tube, to this add 100 µl Buffer 2. Add 100 µl Buffer 3 while vortexing. Centrifuge It	Centrifuge at 14,000 RPM at 4^0C for 4 Mins
Step 2	Carefully transfer the supernatant (300 µl) into fresh 1.5 ml microcentrifuge tube and add 300 µl of Buffer 4. Mix by vortexing. Transfer 600ul in the spin column and centrifuge it.	Centrifuge at 10,000 RPM at 4^0C for 1 Min
Step 3	Place the spin column into a clean 2 ml collection tube and add 600 µl of Wash Buffer 1	Centrifuge at 14,000 RPM at 4^0C for 1 Min
Step 4	Place the spin column in a clean 2 ml collection tube and add 600 µl of Wash Buffer 2.	Centrifuge at 14,000 RPM at 4^0C for 1 Min
Step 5 ***OPTIONAL***	Place the spin column in a clean 2 ml	Centrifuge at full speed at 4^0C for 1 Min (dry spin)
Step 6	Place the spin column in a clean 1.5ml microcentrifuge tube and add 50 µl Elution Buffer equilibrated to room temperature (RT). Incubate at RT for 1min. (Centrifuge to Collect Extracted RNA)	Centrifuge at 10,000 RPM at 4^0C for 1 Min

RNA Extraction Protocol (Long Protocol) (Use for higher yield)

Steps	Instructions	Centrifuge
Step 1	Take 200µl of VTM add 150 of Buffer 1, mix by vortexing for 1 minute.	Centrifuge at 8,000 RPM at 4^0C for 8 Mins
Step 2	Transfer 100µl of supernant in a new micro-centrifuge tube. To this add 100 of Buffer 2. Add 100µl of buffer 3 while vortexing. Centrifuge it	Centrifuge at 14,000 RPM at 4^0C for 4 Min
Step 3	Carefully transfer the supernant (300µl) into fresh 1.5 ml micro-centrifuge tube add 300 of buffer 4. Mix by vortexing. Transfer 600 in the spin column and centrifuge it.	Centrifuge at 10,000 RPM at 4^0C for 1 Min
Step 4	Place the spin column into a clean 2 ml collection tube and add 600 of Wash Buffer 1	Centrifuge at 14,000 RPM at 4^0C for 1 Min
Step 5	Place the spin column into a clean 2ml collection tube and 600 of Wash Buffer 2	Centrifuge at 14,000 RPM at 4^0C for 1 Min

Step 6 ***OPTIONAL***	Place the spin column in a 2ml collection tube.	Centrifuge at full speed at 4^0C for 1 Min (dry spin)
Step 7	Place the spin column in a clean 1.5ml micro-centrifuge tube and add 50 Elution Buffer equilibrated to room temperature (RT). Incubate at RT for 1 min.	Centrifuge at 10,000 RPM at 4^0C for 1 Min

Note:

- *Close each spin column to avoid cross-contamination during centrifugation.*
- *Centrifugation is performed at 6000 x g (8000 rpm) to limit microcentrifuge noise. Centrifugation at full speed will not affect the yield or purity of the viral RNA. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.*

Note:

- *Residual Wash Buffer 2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in flow through, containing Wash Buffer 2, contacting the spin column. Removing the spin column and collection tube from the rotor may also cause flow-through to come into contact with the spin column.*

Shipping and storage

Spin columns should be stored dry at room temperature (15-25°C); storage at higher temperatures shuld be avoided. All solutions should be stored at room temperature unless otherwise stated. Under these conditions, spin columns and all buffers and reagents can be stored until the expiration date on the kit box, without showing any reduction in perf ormance.

Intended Use

MDS™ Viral RNA Extraction Kit contains enhancer buffer for optimal RNA extraction, is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. The Viral RNA Extracted using this kit should be used for downstream applications like PCR.

Principle

MDS™ Viral RNA Extraction Kit represent a well-established technology for general-use viral RNA preparation. The kit combines the selective binding properties of a silica-based membrane with the speed of microspin and is highly suited f or simultaneous processing of multiple samples. The sample is first lysed under highly denaturing conditions to inactivate RNases and to ensure isolation of intact viral RNA.

Buffering conditions are then adjusted to provide optimum binding of the RNA t o the membrane, and the sample is loaded onto the spin column. The RNA binds to the membrane, and contaminants are efficiently washed away in 2 steps using 2 different wash buffers. High quality RNA is eluted in a special RNase-fr ee buffer, ready f or direct use or saf e storage. The purified RNA is fr ee of p rotein, nucleases and other contaminants and inhibitors. The special membrane guarantees extremely high recovery of pure, intact RNA in just 25 min without the use of phenol/chlorof orm extraction or alcohol precipitation.

Equipment and Reagents to be supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

1. Ethanol (96–100%)
2. 1.5 ml microcentrifuge tubes
3. Sterile, RNase-free pipette tips (pipette tips with aerosol barriers for preventing cross contamination are recommended)
4. Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)

Preparation of Reagents

Wash Buffer 2: Wash Buffer 2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (100%) as indicated on the bottle. Wash Buffer 2 is stable for 1 year when stored closed at room temperature, but only until the kit expiration date.

Handling the Spin columns:

Owing to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling spin columns to avoid cross-contamination between sample preparations:

1. Carefully apply the sample or solution to the spin column. Pipet the sample into the spin column without wetting the rim of the column.
2. Change pipette tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
3. Avoid touching the column membrane with the pipette tip.
4. After all pulse-vortexing steps, briefly centrifuge 1.5 ml microcentrifuge tubes to remove drops from the inside of the lid.
5. Wear gloves throughout the procedure. In case of contact between gloves and sample, change gloves immediately.

Spin Protocol

1. Close the spin column before placing it in the microcentrifuge. Centrifuge as described.
2. Remove the spin column and collection tube from the microcentrifuge. Place the spin column in a new collection tube. Discard the filtrate and the old collection tube. Please note that the filtrate may contain hazardous waste and should be disposed of properly.
3. Open only one spin column at a time, and take care to avoid generating aerosols.
4. For efficient parallel processing of multiple samples, it is recommended to fill a rack with collection tubes to which the spin columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the spin columns can be placed directly in the microcentrifuge.

Protocol: Purification of Viral RNA (Spin Protocol)

A. Adherent or Suspension Cultured Cells (10^3 to 10^6 cells)

1. Harvest the cells using a method appropriate to the cell type and growth vessel. If trypsin is used for adherent cells harvest, it should be inactivated.
2. Count the cells. Optional: Wash cells with PBS or similar buffer to remove serum and media components.
3. Pellet the cells by centrifugation at 1,000 x g at 4°C for 5 minutes. Carefully aspirate the supernatant.
4. Add 300 µl of Buffer 1 to the cells and vortex mix for 1 full minute.

B. Swabs collected in viral transport medium

1. Take 200 µl 1. of viral transport medium (VTM) containing specimen into a 1.5 ml microcentrifuge tube.
2. Follow the protocol as mentioned in the brochure

Note:

- *Elution buffer has necessary components to prevent microbial growth and contamination with Rnases.*
- *Filter Tips are not supplied with the Kit*
- *Kit allows extraction of viral RNA from Human Sample (Plasma, CSF, Urine, other cell free body fluids and cell-culture supernatants.)*

Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this kit insert. The information contained in this kit insert is based on our research and development work and is to the best of our knowledge true and accurate. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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PATHKITS

MDS™ Viral RNA Extraction Kit

Product Code : **MDS011**
Kit Contents (**500 Reactions**)

Kit Components	Quantity	Packaging
Silica Spin Column	500	Zip Pouch
Micro Centrifugal Tubes	500	Zip Pouch
Lysis Buffer	200 mL	4 x 50 mL
Isopropyl Alcohol (IPA)	200 mL	4 x 50 mL
*Wash Buffer	500 mL	10 x 50 mL
Elution Buffer	30 mL	1 x 50 mL
Carrier RNA Solubilized Form *(Supplied Separately)*	5.0 mL	1 x 15 mL

** Wash Buffer is supplied as a concentrate (10 mL in 50 mL Bottle), Before using for the first time, add 40 mL of ethanol (96-100%) to each bottle. Wash Buffer is stable for 1 year when stored closed at room temperature, but only until the kit expiration date.*

Notes

- Ethanol is not supplied with the kit
- Mix Carrier RNA (Lyophilised Form) with Carrier RNA Solubilization Buffer to prepare Carrier RNA solution. Step applicable only if carrier rna is provided as Lyophilised form
- If your RT PCR KIT is supplied with an exogenous internal control (IC), then the EIC supplied with your RT PCR kit has to be added in step 3 as per chart below.
- If your RT PCR KIT is supplied with an endogenous internal control , then the IC supplied with your RT PCR kit need not be added in step 3 as per chart below.

Shipping and storage

Carrier RNA in lyophilized form can be stored in Room Temp. for one year from the date of manufacturing. Carrier RNA in solubilized form has to be stored at -20°C for one year (Shipped at Room Temp.). Spin columns should be stored dry at room temperature (15-25°C); storage at higher temperatures should be avoided. All solutions should be stored at room temperature unless otherwise stated. Under these conditions, spin columns and all buffers and reagents can be stored until the expiration date on the kit box, without showing any reduction in performance.

Equipment and Reagents to be supplied by User

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Ethanol (96–100%)
- 1.5 ml microcentrifuge tubes
- Sterile, RNase-free pipette tips (pipette tips with aerosol barriers for preventing cross contamination are recommended)
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)

RNA Extraction Protocol

Step 1 (Transfer): Transfer 200µl of viral transport media (after vortexing) into a fresh Micro Centrifuge Tube (MCT), provided with the kit.

Step 2 (Lysis): Add 400µl Lysis Buffer followed by 10 µl carrier RNA into the MCT and mix thoroughly by pipetting.

Step 3 (Bind): Add 400µl Isopropyl Alcohol (IPA) to the samples and mix well. Transfer 500µl of the total solution to the spin column in a 2 ml collection tube. Close the cap and centrifuge at 8000 rpm for 1 min. Repeat with the remaining 500µl of the total solution.

Step 4 (Wash): Carefully open the spin column and add 500µl of Wash Buffer, and centrifuge at 8000rpm for 1 min and discard the filtrate. Repeat step 4 with 500µl of Wash Buffer. Centrifuge (dry spin) at full speed for 1 min.

Step 5 (Elution): Now place the column in clean 1.5ml RNase Free Micro Centrifuge Tube (not provided with kit) and add 60µl of elution buffer. Centrifuge at 8000 rpm for 1 min.

The eluted viral RNA can be used for all applications such as RT-PCR or sequencing.

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ICMR-National Institute for Implementation Research on Non-Communicable Diseases (NIIRNCD), Jodhpur

28-06-2020

M/s MetaDesign Solutions Pvt. Ltd.

Subject : Performance evaluation report of MDS Viral RNA Extraction Kit manufactured by MetaDesign Solutions Pvt. Ltd.

Sir,
We have evaluated the MDS Viral RNA Extraction Kit manufactured by MetaDesign Solutions Pvt. Ltd. The final report is attached for your information.

Yours sincerely

Dr. G.S. Toteja
Director

ICMR-National Institute for Implementation Research on Non-Communicable Diseases (NIIRNCD), Jodhpur

Performance evaluation report for Viral RNA Extraction kit

Name of the kit and manufacturer: MDS Viral RNA Extraction Kit manufactured by MetaDesign Solutions Pvt. Ltd. (Cat. No.:MDS011)

Lot and batch number: 06-01/2020

Application: Isolation of nucleic acid using spin-column based technology for detection of viral RNA by real time PCR.

Kit components: Spin columns, Collection tubes (2ml), Buffer 1, Buffer 2, Buffer 3, Buffer 4, Wash Buffer 1, Wash Buffer 2, Elution buffer.

Sample Panel

1. SARS CoV-2 Positive samples (n=12)(4 High, 4 Med, 4 Low Ct values)
2. SARS CoV-2 Negative samples (n=12)

Results

- Detection of positive samples as positive : 100% concordant
- Detection of negative samples as negative : 100% concordant
- Detection of IC in all samples in the panel : 100% concordant

Conclusion:

100% concordance observed among positive and negative samples
100% detection of internal control

Performance : SATISFACTORY

The company shall not publish or use information related to this evaluation without prior written consent from ICMR-NIIRNCD.

Prof (H) Dr. G.S. Toteja,
Director,
ICMR-NIIRNCD.

For Kit evaluation team

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