PATHKITS

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

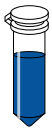
NOTE:

- *Ethanol is not supplied with the kit*
- *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

1

Take 100 µL of Viral Transport Medium (VTM) containing specimen.



Centrifuge | 3000 rpm | 4°C | 5 mins

2

To the cells pellet add 150 µL of Buffer 1, mix by vortexing for 1 minute.

Centrifuge | 6000-8000 rpm | 4°C | 10 mins

3

Take 100 µl of supernatant in a fresh 1.5 ml micro-centrifuge tube. To this add equal volume (100 µl) of preheated (at 70°C) Buffer 2. Subsequently, add 4 µl of internal control (IC).

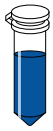
4

Mix thoroughly and then add 100 µl of Buffer 3. Mix by vortexing.

Centrifuge | 14000 rpm | 4°C | 5 mins

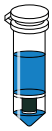
5

Carefully transfer the supernatant (300 µl) into fresh 1.5 ml micro-centrifuge tube and add 300 µl of Buffer 4. Mix by vortexing.



6

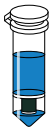
Carefully transfer 600 µl of the solution to the spin column (in a 2 ml collection tube).



Centrifuge | 8000 rpm | 4°C | 2 mins

7

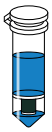
Place the spin column into a clean 2 ml collection tube and add 600 µl of Wash Buffer 1.



Centrifuge | 8000 rpm | 4°C | 2 mins

8

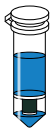
Place the spin column in a clean 2 ml collection tube and add 600 µl of Wash Buffer 2.



Centrifuge | 8000 rpm | 4°C | 2 mins

9

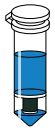
Place the spin column in a clean 2 ml collection tube.



Centrifuge | 12500 rpm | 4°C | 2 mins (Dry Spin)

10

Place the spin column in a clean 1.5ml micro-centrifuge tube and add 50 µl Elution Buffer equilibrated to room temperature (RT). Incubate at RT for 1min.



Elute RNA

Centrifuge | 8000 rpm | 4°C | 2 mins

11

Collect extracted

RNA

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 performance.

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.

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 for 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 to 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-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases and other contaminants and inhibitors. The special membrane guarantees extremely high recovery of pure, intact RNA in just 20 min without the use of phenol/chloroform 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 500 µl of viral transport medium (VTM) containing specimen into a 1.5 ml microcentrifuge tube.
2. Centrifuge at 1,000 x g at 4°C for 5 minutes. Discard the supernatant.
3. To the cell pellet 150 µl of Buffer 1 and vortex mix for 1 full minute.

Extraction Protocol

1. Centrifuge the prepared cells as per the protocol described above at 5000 x g for 10mins.
2. Take 100 µl supernatant in a fresh 1.5 ml microcentrifuge tube, to this add equal volume (100 µl) of preheated (at 70 degrees) Buffer 2. Mix thoroughly and then add 100 µl of Buffer 3, while vortexing.
3. Centrifuge the lysed cells at 14,000 rpm at4°C for 5 mins.
4. Carefully aspirate the supernatant (300 µl) into fresh 1.5 ml microcentrifuge tube and add 300 µl of Buffer 4. Mix by vortexing.
5. Carefully apply 600 µl of the solution from step 4 to the spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g for 1 min. Place the spin column into a clean 2 ml collection tube, and discard the tube containing the filtrate.

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.*

6. Carefully open the spin column, and add 600 µl of Wash Buffer 1. Close the cap, and centrifuge at 6000 x g for 1 min. Place the spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
7. Carefully open the spin column, and add 600µl of Wash Buffer 2. Close the cap and centrifuge at full speed (20,000 x g) for 2 min.

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. In such cases, it is recommended to follow step 8 before final elution.*

8. Place the spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
9. Place the spin column in a clean 1.5 ml microcentrifuge tube. Discard the old collection tube containing the filtrate. Carefully open the spin column and add 50 µl Elution Buffer equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min.
10. Centrifuge at 6000 x g for 1 min.
    A single elution with 50 µl Elution Buffer is sufficient to elute at least 90% of the viral RNA from the spin column. Viral RNA is stable for up to 1 year when stored at -15°C to -90°C.

Disclaimer:

MetaDesign Solutions: : Plot 28, 29 Sector 18(P) Udyog Vihar Phase IV,
Gurgaon -122015, India Customer care No.: +91-730-342-9198
Email: info@pathkits.com