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Viral DNA/RNA Purification and Manual Isolation of Viral DNA/RNA in Microfuge Tubes (1.5 or 2.0 ml) SKU T4010 V.1

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We use this protocol and it's working

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Abstract

This protocol details the purification and isolation of viral DNA and RNA in microfuge tubes.

Guidelines

Important Notes about Viral DNA and RNA Purification

Copurification of Viral Nucleic Acid and Carrier RNA:

- Viral nucleic acid and carrier RNA are purified in parallel. Depending on the input sample, viral nucleic acid yield may be relatively low and is often too low to be determined spectrophotometrically or fluorometrically. RT-qPCR/qPCR is recommended to determine viral RNA and DNA yields.
- Carrier RNA may account for much of the purified nucleic acid and its recovery can be quantified spectrophotometrically.
- To remove unwanted DNA or RNA from a purified sample, further treatment with the appropriate nuclease is required (not supplied).

Procedure Notes

This optimized viral nucleic acid extraction procedure employs a sample lysis step followed by a simple bind-wash-elute process. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Perform all steps at room temperature unless directed otherwise.

▪ **Sample Lysis and Binding of Nucleic Acid to Beads:**

Samples are lysed using Proteinase K and a user-prepared lysis buffer/bead mixture containing lysis buffer, carrier RNA, isopropanol (user supplied), and magnetic beads. The buffer conditions, and thorough mixing, promote binding of extracted nucleic acid onto silica-coated beads.

▪ **Magnetic Bead Collection Times:**

The superparamagnetic properties of the silica-coated beads result in a fast magnetic response, contributing to ease of handling during use. Recommended bead collection times are listed for the relevant protocol steps; however, bead collection times may vary depending on the sample type and magnet used.

▪ **Wash Steps:**

Following the binding of viral nucleic acid onto silica-coated beads, beads are subjected to washes with mixing to remove residual contaminants, including proteins and salts. Two user-prepared wash buffers are utilized in three wash steps. Users prepare Viral DNA/RNA Wash Buffer for the first wash step, and 80% ethanol for the second and third wash steps. Recommended wash volumes vary and are listed in each protocol.

A thermal mixer or vortex mixer can be used to mix bead washes in microcentrifuge tubes. For bead washes in deep well plates, a thermal mixer or plate shaker can be used. Using KingFisher Flex automation, the magnetic head and tip comb are utilized for mixing.

▪ **Bead Drying:**

Following a third wash step, the magnetic beads are air dried. Recommended bead drying times are listed in the protocol. The drying time can be extended if any visible droplets of liquid remain, or if the beads appear wet; however, overdrying of beads is not recommended as yield may be reduced.

■ **Heated Elution:**

Nucleic acid is eluted from dried beads in a heated elution step. Nuclease-free water is added to the beads and samples are incubated at 65°C with mixing. Beads are then collected on a magnet, and the eluate is carefully transferred to a new tube or plate, without disturbing the beads. Purified nucleic acid can be stored on ice for short-term storage or at -80°C for long-term storage.

Considerations for Maximizing Viral RNA Recovery:

Viral DNA and RNA are copurified using this optimized viral nucleic acid extraction protocol. To maximize viral RNA recovery, keep the following principles in mind:

- Successful RNA extraction and purification are greatly influenced by the type of sample and the user's ability to maintain RNA integrity during sample collection, storage, and processing.
- RNases are stable and difficult to inactivate, therefore, care must be taken when handling samples during and after preparation of RNA. Plasticware and glassware in direct contact with RNA-containing samples should be RNase-free. Gloves should be worn at all times when handling samples and kit components. Frequent glove changes are encouraged. Bench and equipment surfaces should be clean and can be decontaminated before work using commercially available cleaners such as RNaseZAP™.
- Elution with nuclease-free water is standard, but for samples that will be stored for use later, EDTA can be added to 0.1–1.0 mM to limit degradation due to magnesium-requiring nucleases. Alternatively, elution with slightly alkaline TE can be employed.
- Avoid repeated freeze-thaw cycles of purified RNA. Aliquots should be made consistent with downstream needs.

Important Notes Before You Begin Manual Isolation of Viral DNA/RNA in Microfuge Tubes (1.5 or 2.0 ml):

- Review Reagent Preparation section.
- Store Proteinase K at  -20 °C upon receipt.
- **Prepare Monarch Carrier RNA based on kit size used:** Add 125 µl (NEB #T4010S) or 750 µl (NEB #T4010L/X) nuclease-free water, invert or pipette to mix, and transfer to an RNase-free microfuge tube. Keep  On ice . Prepare single-use aliquots and store at  -20 °C . Avoid multiple freeze-thaw cycles.
- **Prepare 80% ethanol:** 80% ethanol should be prepared fresh using 100% absolute ethanol (user supplied) and nuclease-free water (user supplied). Prepare  1 mL of 80% ethanol per reaction and add overage.
- Perform all steps at  Room temperature unless directed otherwise.

Materials

Buffer preparation

Viral DNA/RNA Wash Buffer:

	A	B
	Volume per reaction	
	a. Combine the following:	
	Monarch Buffer BX	167 µl
	Nuclease-free Water	83 µl
	b. Vortex to mix and then add:	
	Isopropanol	250 µl
	c. Vortex to mix	
	Total Volume	500 µl

Lysis Buffer Bead Mix:

	A	B
	Volume per reaction	
	a. Combine the following:	
	Monarch StabiLyse DNA/RNA Buffer	200 µl
	Monarch Carrier RNA	1 µl
	b. Vortex to mix and then add:	
	Isopropanol	200 µl
	c. Vortex to mix and then add:	
	Monarch Mag Beads M1	20 µl
	d. Gently vortex to mix	
	Total Volume	421 µl



Procedure Notes

1

Note

"Please review the important information under the "Guidelines & Warnings".

Buffer Preparation

2

Note

Starting Material Notes:

This protocol has been optimized for use with  200 μL saliva or a respiratory swab sample collected in viral transport media (VTM). For samples < 200 μL , the sample volume should be adjusted to 200 μL with VTM or PBS before processing.

Prepare fresh viral DNA/RNA wash buffer in a user-supplied tube or bottle (free of nucleases) according to the table. Add components in order, as listed. Prepare up to 15% excess to ensure a sufficient volume is available for each reaction.

3 Prepare lysis buffer bead mix immediately before use, according to the table.

3.1 Vortex magnetic beads to form a homogeneous solution before use.

3.2 Add components in order, as listed.

3.3 For a master mix, prepare up to 15% excess to ensure a sufficient volume of buffer/bead mix is available for each reaction.

3.4 Store lysis buffer bead mix at  Room temperature . Periodically invert or vortex to keep beads in suspension.



Viral DNA/RNA Wash Buffer:

A	B
Volume per reaction	
a. Combine the following:	
Monarch Buffer BX	167 µl
Nuclease-free Water	83 µl
b. Vortex to mix and then add:	
Isopropanol	250 µl
c. Vortex to mix	
Total Volume	500 µl

Lysis Buffer Bead Mix:

A	B
Volume per reaction	
a. Combine the following:	
Monarch StabiLyse DNA/RNA Buffer	200 µl
Monarch Carrier RNA	1 µl
b. Vortex to mix and then add:	
Isopropanol	200 µl
c. Vortex to mix and then add:	
Monarch Mag Beads M1	20 µl
d. Gently vortex to mix	
Total Volume	421 µl

Sample Lysis

15m

4 Add  5 µL Proteinase K to each 1.5 ml microfuge tube.





5 Add  200 μL sample (e.g., saliva or nasal swab in VTM) to each tube, and pipette thoroughly to mix.



6 Incubate tubes at  Room temperature for  00:15:00 .

15m



7 Gently vortex lysis buffer bead mix before adding  421 μL to each sample tube. Pipette gently but thoroughly to mix.



Viral Nucleic Acid Purification (Bind, Wash, Elute)

35m

8 Bind nucleic acid to beads:

5m

Mix samples in a thermal mixer for  2000 rpm, 00:05:00 .



8.1 Spin tubes briefly in a benchtop mini centrifuge to collect liquid at the bottom of the tube.

8.2 Place tubes on magnet for  00:03:00 .

3m

8.3 With tubes on the magnet, carefully remove and discard the supernatant.

9 Wash beads:

9.1 Remove tubes from the magnet and add  500 μL viral DNA/RNA wash buffer.



9.2 Mix samples in a thermal mixer for  2000 rpm, 00:01:00 (or vortex mix,  00:00:05).

1m



9.3 Spin tubes briefly in a benchtop mini centrifuge.



9.4 Place tubes on the magnet for 00:03:00 .

3m

9.5 With tubes on the magnet, carefully remove and discard the supernatant.

9.6 Repeat wash steps 9.1–9.5, washing beads with 500 μL 80% ethanol.



9.7 Repeat wash steps 9.1–9.5 for a second wash with 500 μL 80% ethanol.



9.8 Spin tubes briefly in a benchtop mini centrifuge.

9.9 Place tubes on the magnet for 00:03:00 .

3m

9.10 With tubes on the magnet, carefully remove and discard any residual supernatant.

10 **Dry beads:**

10m

Air-dry beads for 00:05:00 . If visible liquid droplets remain in the tube or on the bead pellet, extend the drying time up to 00:10:00 .

Note

(During this bead drying step, set the thermal mixer to 65 °C).

11 **Elute nucleic acid:**

11.1 Remove tubes from the magnet and add 33 μL – 100 μL nuclease-free water.

11.2 Mix samples in a thermal mixer at 2000 rpm, 65°C, 00:05:00 .

5m



5m

- 11.3 Place tubes on the magnet for 00:05:00 .
- 11.4 Transfer the supernatant (i.e., eluted nucleic acid) to a nuclease-free microfuge tube.
- 11.5 Place eluate On ice for immediate use or at -80 °C for long-term storage.