Apr 18, 2024

Small volume viral RNA extraction using MagMAX Viral RNA Isolation Kit

DOI
dx.doi.org/10.17504/protocols.io.81wgbzpyogpk/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.81wgbzpyogpk/v1

Protocol Citation: Erika Bujaki, Joyce Akello, Alex Shaw, Catherine Troman, khurshida, arshady, alammu, Nick Grassly, Javier Martin 2024. Small volume viral RNA extraction using MagMAX Viral RNA Isolation Kit. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.81wgbzpyogpk/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 11, 2024

Last Modified: April 18, 2024

Protocol Integer ID: 98057

Funders Acknowledgement:
Bill and Melinda Gates Foundation
Medicines and Healthcare Products Regulatory Agency

Abstract

This protocol describes the viral nucleic acid recovery and purification from stool suspensions. The method utilises the magnetic bead based MagMAX ™ Viral RNA Isolation Kit to purify nucleic acid from 300 µL of sample. To perform manual extractions follow Workflow A, for automated extraction on King Fisher Duo Prime follow steps in Workflow B, and for automated extraction on King Fisher Flex follow Workflow C.

Materials

Reagents

- Ethanol (96-100%)
- Isopropanol (100%)
- MagMAX Viral RNA Isolation Kit (AM1939). Contains sufficient reagents to isolate RNA from approximately 65 samples (see table below)

Amount		Component	Storage
50		Processing Tubes	room temp
44	mL	Lysis/Binding Soln Concentrate See step 2. on page 10 before use	room temp†
36	mL	Wash Solution 1 Concentrate (Add 12 mL 100% isopropanol before use)	room temp
55	mL	Wash Solution 2 Concentrate (Add 44 mL 100% ethanol before use)	4°C or room temp
5	mL	Elution Buffer	4°C or room temp
550	µL	RNA Binding Beads	4°C†
110	µL	Carrier RNA	–20°C
550	µL	Lysis/Binding Enhancer	–20°C

† Do not freeze these kit components.

- Individual components of the kit can also be purchased separately in larger volumes (as shown with product codes in table below)

	A	B	C	D
	Amount	**Component**	**Product code**	**Storage**
	100 mL	MagMAX‰ Lysis/Binding Solution Concentrate	AM8500	Room temp
	205 mL final volume	Wash Solution 1 Concentrate (Add 70mL 100% isopropanol before use)	AM8504	Room temp
	200 mL final volume	Wash Solution 2 Concentrate (Add 160 mL 100% Ethanol before use)	AM8640	Room temp
	10 mL	MagMAX‰ Total RNA Elution Buffer	A41043	Room temp

	A	B	C	D
	500 µL	Carrier RNA	4382878	-20 °C
	1.8 mL	DNA Binding Beads (same as RNA binding beads in AM1939 kit)	4489112	4 °C
	5 x 1.25 mL	Recombinant Proteinase K Solution (20 mg/mL) (Same as Lysis/Binding Enhancer in AM1939 kit)	AM2548	-20 °C

Equipment

- Biological Safety Cabinet II (Class II BSC)
- PPE
- Magnetic stand accommodating 1.5-2 mL centrifuge tubes / King Fisher Duo Prime equipped with 12 tip-magnetic head / King Fisher Flex equipped with 96 tip deep well magnetic head
- Pipettes and sterile, filtered, RNAse-free pipette tips
- 1.5 mL microcentrifuge tubes (the use of 1.5 mL Eppendorf DNA LoBind tubes or Processing Tubes included in the extraction kit is recommended)
- 15 mL / 50 mL centrifuge tubes for preparing Lysis/Binding Solution
- Tube racks
- Vortex/rotator/thermomixer accommodating 1.5-2 mL centrifuge tubes
- Centrifuge accommodating 1.5-2 mL tubes
- Plasticware for the use of King Fisher Duo Prime equipment with 12-tip magnet, as shown in Appendix 2
- Plasticware for the use of King Fisher Flex equipment with 96-tip magnetic head, as shown in Appendix 3

Reagent Preparation

7m

1 Wash Solution 1

1m

1.1 Add indicated volume of 100% Isopropanol to the bottle of Wash Solution 1 Concentrate.

1.2 Mix well by inverting at least 5 times and mark bottle to indicate that the alcohol was added.

2 Wash Solution 2

1m

2.1 Add indicated volume of 100% ethanol to the bottle of Wash Solution 2 Concentrate.

2.2 Mix well by inverting at least 5 times and mark the bottle to indicate that ethanol was added.

Note

Note: Taking an aliquot of the prepared Wash Solutions is recommended to avoid potential contamination if they are to be used on multiple occasions.

3 Lysis/Binding Solution

3m

Combine the components listed below in the order indicated. Prepare enough reagents for the number of samples extracted that day, including controls, with adding extra 10% for pipetting loss.

Note

Note: Prepare the Lysis/Binding Solution on the day it will be used. Kept at room temperature with a tightened lid.

3.1 Add Carrier RNA to Lysis/Binding Solution Concentrate according to the table below, and mix briefly

A	B
Reagent	**Per sample**
Lysis/Binding Soln. Concentrate	300 µl
Carrier RNA	1.5 µl

3.2 Add 100% Isopropanol and mix well by vortexing.

A	B
Component	**Per sample**
100% Isopropanol	300 µl

4 Bead Mix 2m

Note

Prepare the bead mix on the day it will be used. Bead mix can be stored on ice until it is needed for up to 4 hours. Avoid freezing the mixture as it damages the magnetic beads.

4.1 Vortex the nucleic acid binding beads well to ensure that the beads are fully resuspended.

4.2 Combine the components that are listed below:

A	B
Component	**Volume per sample**

	A	B
	Nucleic Acid Binding Beads	10 µl
	Lysis/Binding ENHANCER	10 µl
	Total volume	20 µl

4.3 Mix well by vortexing and place the prepared bead mix on top of ice or in the fridge until it is needed, but avoid freezing as it destroys its properties.

RNA extraction - Workflows A,B,C

1h 30m

5 Manual extraction – Workflow A

1h 10m

5.1 Prepare the lysate:

10m

For each sample:

1. Set up and label 1.5 ml Eppendorf DNA LoBind centrifuge tubes, then aliquot 600 µl of the lysis/binding solution (supplemented with carrier RNA and 100% Isopropanol - see Reagent preparation section) into each tube.

2. Transfer 300 µl of the stool suspension supernatant to the labelled tubes containing the Lysis/Binding Solution (supplemented with carrier RNA and 100% Isopropanol).

Note

When adding sample, check and confirm the sample ID. Immerse pipette tips slightly in the Lysis/Binding Solution to prevent creating aerosols and rinse pipette tip. Keep all other tubes closed to avoid cross contamination.

3. Mix gently by vortexing for 30s and spin briefly to collect tube content.

5.2 Bead capture and washes:

50m

4. Add 20 µl of prepared bead mix to each sample tube containing the lysed sample solution.

Note

Mix bead solution by pipetting to avoid settling down of the magnetic beads. Use a new tip for each addition to minimise bead loss and rinse the tip gently a few times to ensure full volume is transferred.

5. Mix tubes thoroughly at gentle speed for 4 min to fully lyse viruses and bind RNA to beads.

Note

A shaker or rotator mixer can be used for this step or tubes can be repeatedly rotated or gently vortexed manually. It is important to achieve sufficient mixing in this step with a visibly homogenous coloured mixture throughout to ensure efficient lysis and bead binding.

6. Centrifuge tubes briefly to collect content, then place on magnet and leave for at least 2 minutes to allow for bead capture to complete. Beads should form a pellet against the magnet.

Note

Pellets may also be smeared on the tube's wall as well as forming a compact body. Twisting the tube gently help to make the pellet more compact if needed.

7. Carefully aspirate and discard supernatant without disturbing the beads.

8. It is important to remove the lysis supernatant fully, so a brief centrifugation before collecting the remaining supernatant might be necessary.

9. Remove tubes from magnetic stand and place in tube rack for washing with Wash Solution 1.

10. Add 300 µl Wash Solution 1 to each sample and vortex at moderate speed for 30s.

Note

Pellets do not necessarily get fully resuspended in all samples.

11. Centrifuge briefly to collect tube content.

12. Capture beads on magnet until mixture becomes clear, indicating full capture.

13. Carefully aspirate and discard supernatant.

14. Repeat steps 9-13 one more time to complete two washes with Wash Solution 1.

15. Remove tube from magnetic stand and place in tube rack for washing with Wash Solution 2.

16. Add 450 µl Wash Solution 2 to each sample and vortex at moderate speed for 30s.

Note

Beads can appear granular during washing steps with Wash Solution 2.

17. Centrifuge briefly to collect tube content.

18. Capture beads on magnet for 2 mins or until mixture becomes clear, indicating full capture.

19. Carefully aspirate and discard supernatant.

20. Repeat steps 15-19 to complete two washes with Wash Solution 2.

Note

It is important to completely remove the supernatant after the second wash to avoid inhibition in downstream applications.

5.3 Drying the beads and elution: 10m

21. Centrifuge briefly and remove any residual solution with a small volume fine-tipped pipette, without disturbing the pellet.
22. Dry the beads by leaving the tube open for 2 minutes to allow any remaining alcohol to evaporate.

Note

Avoid overdrying the beads to the point of the pellet cracking and crumbling as this may lower the efficieincy of nucleic acid recovery.

23. Add 50 µL Elution Buffer to each sample and shake/vortex vigorously for 4 min to fully resuspend the pellet.

24. Centrifuge briefly to collect tube content.

25. Capture the beads on the magnet as before and collect supernatant containing the purified RNA in labelled containers and keep on ice for immediate use or store frozen until needed.

Note

Open tubes before placing on the magnet to avoid tube content flipping on sidewall. When this happens, remove tube and centrifuge briefly before replacing on the magnet.

Note

If beads are accidentally collected, return fluid from the pipette tip, and try again once supernatant is fully clear.

Note

Pellet might be smeared around the bottom of the tube instead of forming a compact pellet against the magnet. Look into the tube from above to check if eluate has fully cleared. Aim to collect RNA from a central position without touching the pellet.

6 Automated extraction using King Fisher Duo Prime – Workflow B

54m

MVRI_DUO_SV_300ul.bdz 3KB

Read the King Fisher Flex instrument manual for installation and operating instructions in its entirety before operating the magnetic particle processor.

Please find attached the associated King Fisher Duo Prime protocol for importing into your equipment.

Plasticware for small volume sample extractions on King Fisher Duo Prime equipped with 12-tip magnetic head.

	A	B
	Item	**ThermoFisher product code**
	KingFisher deep-well 96 plate (50)	95040450

	A	B
	King Fisher Duo Combi Pack for 96 DW Plate All plasticware for extraction of 8 plates (96samples)	97003530
	KingFisher Duo cap for elution strip (40)	97003540
	KingFisher Duo elution strip (40)	97003520
	KingFisher 96 KF plate	97002540
	KingFisher 12 tip comb for 96 deep-well plate (50)	97003500

6.1 Preparing 96 deep well plate: 20m

Note

Label your plates and elution strips if using more than one.

Set up plate by loading the required reagents into the appropriate positions as shown in table below:

	Plate row	Plate type	Reagent	Volume per well (µl)
Sample Lysis/Binding	A	MME -96 deep well plate	Lysis/Binding solution +cRNA+ 100% Isopropanol	600
			clarified sample	300
			Bead mix	20
First wash 1	B	MME -96 deep well plate	Wash solution 1	300
Second wash 1	C	MME -96 deep well plate	Wash solution 1	300
First wash 2	D	MME -96 deep well plate	Wash solution 2	450
Second wash 2	E	MME -96 deep well plate	Wash solution 2	450
	F			
	G			
12-Tip comb	H	MME-96 deep well plate	MME-96 deep well tip comb in standard plate	
Elution		Elution strip tube	50 µl Elution buffer / nuclease free water	

1. Aliquot 600 µl of the Lysis/Binding Solution supplemented with carrier RNA and 100% Isopropanol (see step 1 and 2 in Reagent preparation) to the top row (row A) of the 96 deep-well plate.

2. Transfer 300 µl of the sample to the same top row (row A) of the 96 deep-well plate containing the Lysis/Binding Solution. Mix by gently pipetting up and down in the 96 deep-well plate a few times. (Discard tube containing the punches).

Note

When adding sample, check and confirm the sample ID. Immerse pipette tips slightly in the lysis/binding solution to prevent creating aerosols and rinse pipette tip. Keep all other tubes closed to avoid cross contamination.

3. Add 20 µl of prepared bead mix to row A of the 96 deep well plate containing the lysed sample solution using a new tip for every addition and rinsing it with the sample solution.

Note

Mix bead solution by pipetting to avoid settling down of the magnetic beads. Use a new tip for each addition and rinse the tip gently a few times to ensure full volume is transferred.

4. Set up plate by aliquoting the required reagents into the appropriate positions as shown in table below:

5. Add 300 µl of prepared wash solution 1 to rows B and C of the 96 deep well plate.

6. Add 4500 µl of prepared wash solution 2 to rows D and E of the 96 deep well plate.

7. Place a 12-tip comb in a 96 deep well plate in Row H.

8. Add 50 µl of Elution buffer/ nuclease free water to the elution strip tube.

6.2 Setting up and running the King Fisher Duo Prime: 31m

9. Check to confirm that the KingFisher Duo Prime is set up with 12-tip magnet and heating block.

Follow the below steps to change the magnetic head on the King Fisher Duo Prime if necessary:

- Select and start Change Magnetic Head protocol in Maintenance protocols in the device menu. (This will position the magnet to be accessible.)
- Unscrew and remove the screws holding the magnetic head in place and lift the magnetic head to take it out.
- Replace the required magnetic head and tighten the screws to hold it in place.
- Remember to also change the heating blocks as the machine doesn't give a prompt to do so!
- Run Check 12 tip protocol with a dummy test plate containing the tip comb in the required row to ensure the right positioning. Unload the test plate.

10. Load the prepared sample plate onto the King Fisher Duo Prime as prompted, ensuring the right orientation by matching the A1 marking on the turntable. Ensure that the plate is lying completely flat.

11. Place the prepared elution strip into the device in the metal rack next to the loaded plate and use the fold over lock on the elution block to secure it in place. Use the position of the small round hole on the elution strip to match the red conical protrusion on the rack to ensure the right orientation. Both the plate and the elution strip should be on the same side of the turntable.

12. Select the MVRI_DUO_SV_300ul Protocol and press start.

13. Close the front lid while the KingFisher is running.

6.3 Un-loading the device: 3m

14. After completion of the run, a final prompt will appear. "Unload RNA plate and RNA Elution Strip".

15. Unload the elution strip containing the RNA, cap and place the elution strip containing the RNA on ice.

16. Remove the sample plate from the device then press the “Check Mark”. Discard the plate and content following the appropriate laboratory procedure. Wipe clean equipment, then switch on UV light for disinfection.

17. Transfer eluted viral RNA to labelled containers and keep on ice for immediate use or store frozen until needed.

7 **Automated extraction using King Fisher Flex – Workflow C** MVRI_Flex_SV_300ul.bdz 3KB 1h 30m

Read the King Fisher Flex instrument manual for installation and operating instructions in its entirety before operating the magnetic particle processor.

Please find attached the associated King Fisher Flex protocol for importing into your equipment.

Plasticware for small volume sample
extractions on King Fisher Flex equipped with 96-tip magnetic head.

	A	B
	Item	**ThermoFisher product code**
	KingFisher deep-well 96 plate (50)	95040450
	KingFisher 96 tip comb for deep-well magnets (100)	97002534
	KingFisher 96 KF plate For tip comb placement and eluate storage (48)	97002540

7.1 Preparing sample plate 30m

1. Aliquot 600 µl of the Lysis/Binding solution supplemented with carrier RNA and 100% Isopropanol (see step 3 in Reagent preparation) to a 96 deep-well plate marked as Sample plate.

2. Add 300 µl of samples to the 96 deep-well plate containing the Lysis/Binding Solution. Mix by gently pipetting up and down a few times. (Discard tube containing the punches).

Note

When adding sample, check and confirm the sample ID. Immerse pipette tips slightly in the lysis/binding solution to prevent creating aerosols and rinse pipette tip. Keep all other tubes closed to avoid cross contamination.

7.2 Setting up the processing plates and running the extraction program

55m

3. Add 20 µl of prepared bead mix to the wells of the Sample plate containing the Lysed sample solution using a new tip for every addition and rinsing it with the sample solution to prevent loss of beads.

4. Label and prepare the processing plates according to the table below:

Note

Using an automated multidispenser pipette with appropriate combitips tips or the use of multichannel pipettes is recommended for the following steps for maintaining accuracy with solutions containing alcohols and to reduce repetitive strain. Adjust volume and use a new tip for each solution.

Plate	Plate type	Reagent	Volume per well
Sample plate	96 deep well plate	Lysis/Binding solution +cRNA+ 100% Isopropanol	600
		clarified sample	300
		Bead mix	20
First wash 1 (FW1)	96 deep well plate	Wash solution 1	300
Second wash 1 (SW1)	96 deep well plate	Wash solution 1	300
First wash 2 (FW2)	96 deep well plate	Wash solution 2	450
Second wash 2 (SW2)	96 deep well plate	Wash solution 2	450
Elution	96 well standard plate	Elution buffer / nuclease free water	50
Tip comb plate	96 well standard plate	Tip comb in standard plate	

5. Add 300 µl of prepared wash solution 1 to the 96 deep well plates marked FW1 and SW1.

6. Add 450 µl of prepared wash solution 2 of the 96 deep well plates marked FW2 and SW2.

7. Add 50 µl of nuclease free water to wells of the 96-standard plate marked Elution.

8. Place a 96 tip comb in a 96-standard plate.

9. Check to confirm that the instrument is set up with 96 deep-well magnetic head and 96 deep-well heat block. Select the MVRI_Flex_SV_300ul protocol on the equipment and load the plates onto the King Fisher Flex as directed, then start the protocol.

10. Close the front lid of the device.

7.3 Un-loading the device: 5m

11. After completion of the run, a final prompt will appear. “Unload plate containing the RNA”.

12. Transfer eluted viral RNA to labelled containers and keep on ice for immediate use or store frozen until needed.

13. Empty and wipe clean equipment. Dispose of processing plates and their contents by following standard laboratory processes.