Large volume viral RNA extraction using MagMAX Viral RNA Isolation Kit

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MAR 20, 2024

OPEN ACCESS

Protocol Citation: Erika Bujaki, Thomas Wilton, Dimitra Klapsa, Alison Tedcastle, Joyce Akello, Nick Grassly, Javier Martin 2024. Large volume viral RNA extraction using MagMAX Viral RNA Isolation Kit. **protocols.io** https://protocols.io/view/large-volume-viral-rna-extraction-using-magmax-vir-daxv2fn6

MANUSCRIPT CITATION:

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

Created: Mar 20, 2024

ABSTRACT

This method describes large volume nucleic acid purification from sewage concentrates starting with 1.2 mL of sample using MagMAX ™ Viral RNA Isolation Kit. To perform automated extraction, follow steps in Workflow A, and for manual extractions follow Workflow B diverging after the initial sample processing with Lysis/Binding solution. Appendix 1 contains pictures providing a visual guide to manual magnetic beads based nucleic acid extraction.

Last Modified: Mar 20, 2024

PROTOCOL integer ID: 96981

Keywords: sewage testing, nucleic acid extraction, large volume extraction, magnetic bead extraction, environmental testing, viral RNA extraction

Funders Acknowledgement:
Medical and Healthcare products Regulatory Agency
Bill and Melinda Gates Foundation
Grant ID: INV-024477

MATERIALS

Equipment

- Biological Safety Cabinet II (Class II BSC), PPE
- King Fisher Duo Prime equipped with 6 tip-magnetic head / Magnetic stand accommodating 5/15 mL centrifuge tubes
- Pipettes and sterile, filtered, RNAse-free pipette tips
- 1.5 microcentrifuge tubes (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
- 5 mL centrifuge tubes (Eppendorf DNA LoBind tubes are ideal and they have the same diameter as 15 mL centrifuge tubes, fitting in centrifuge rotors and racks.)
- Tube racks
- Vortex/rotator/thermomixer accommodating 5/15 mL centrifuge tubes
- Centrifuge accommodating 5/15 mL tubes

Reagents

- Ethanol (96-100%)
- Isopropanol (100%)
- MagMAX Viral RNA Isolation Kit (AM1939). - Contains sufficient reagents to isolate RNA from approximately 16 large volume 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 in table below):

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
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

Note

Individually purchased components will vary in the number of extractions they can cover, so keep track of usage for each.

1 Reagent preparation

1.1 Wash Solution 1

- Add indicated volume of 100% Isopropanol to the bottle of Wash Solution 1 Concentrate.
- Mix well by inverting at least 5 times and mark bottle to indicate that the alcohol was added.

1.2 Wash Solution 2

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

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

Note

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

1.3 Lysis/Binding Solution

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

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

	A	B
	Reagent	**Volume per sample**
	Lysis/Binding Soln. Concentrate	1.2 ml
	Carrier RNA	6 µl

- Add 100% Isopropanol and mix well by vortexing.

	A	B
	Component	Volume per sample
	100% Isopropanol	1.2 ml

1.4 Bead Mix

Combine the components that are listed below. Prepare enough mixture for the number of samples extracted that day with allowing extra for pipetting loss.

Note

Prepare the bead mix on the day it will be used. Bead mix can be stored in the fridge or on top of ice until it is needed for up to 4 hours. Avoid freezing the mixture as it damages the magnetic beads. Be careful with using cold racks as they can cause accidental freezing of tube content.

- Vortex the nucleic acid binding beads well to ensure they are fully resuspended.

	A	B
	Component	**Volume per sample**
	Nucleic Acid Binding Beads	30 µl
	Lysis ENHANCER/ Proteinase K	30 µl
	Total volume	60 µl

- Mix well by vortexing and store appropriately until needed.

2 Sample Processing

2.1 Prepare the lysate in 5mL or 15mL centrifuge tubes

For each sample:

- Set up and label 5ml Eppendorf tubes, then aliquot 2.4 ml of the lysis/binding solution (supplemented with carrier RNA and 100% Isopropanol - see step 9 and 10 in Reagent preparation) into each tube.
- Add 1.2 ml of the samples to the tubes containing the Lysis/Binding solution.

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Note

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When adding sample, immerse pipette tips slightly in the Lysis/Binding solution to prevent creating aerosols and rinse pipette tip.

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

3 Workflow A - Automated extraction

3.1 Setting up and running the 24 deep well plates

1. Label two 24 deep-well plates as Plate 1 and Plate 2.
2. Transfer the 3.6 ml of sample/lysis mixture to row A of plate 1.
3. Add 60 µl of prepared Bead Mix to wells in row A of the 24 deep well plate 1 containing the lysed sample solution using a new tip for every addition and rinsing it with the sample solution to prevent loss of beads.

Note

A vortex mixer, shaker or rotator mixer can be used for this step or tubes can be repeatedly rotated 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.

4. Centrifuge tubes briefly to collect content.

Plate	Position	Plate type	Reagent	Volume per well
Sample plate	Plate 1 Row A	MME -24 deep well plate	Lysis/Binding solution +cRNA+ 10% Isopropanol	2.4 ml
			clarified sample	1.2 ml
			Bead mix	60 µl
First wash 1	Plate 1 Row B	MME -24 deep well plate	Wash solution 1	900 µl
Second wash 1	Plate 1 Row C	MME -24 deep well plate	Wash solution 1	900 µl
6-Tip comb	Plate 1 Row D	Tip comb	MME-24 deep 6 well tip comb	
First wash 2	Plate 2 Row A	MME -24 deep well plate	Wash solution 2	1350 µl
Second wash 2	Plate 2 Row B	MME -24 deep well plate	Wash solution 2	1350 µl
Elution	Plate 2 Row D	MME -24 deep well plate	Elution buffer / nuclease free water	90 µl

Aliquot the required reagents into the plates according to the table above:

5. Add 900 µl of prepared Wash Solution 1 to rows B and C of Plate 1.
6. Add 1350 µl of prepared Wash Solution 2 to rows A and B of Plate 2.
7. Place a 12-tip comb in a 96 deep well plate in row D of Plate 1.
8. Add 50 µl of Elution Buffer or nuclease free water to row D in Plate 2.
9. Check to confirm that the King Fisher Duo Prime is set up with 6 tip magnet and heating block.
10. Select the program MVRI Duo LV 1200ul and load the two plates using the A1 markings as prompted. The two plates will be on opposing sides of the turning platform in the processor.
11. Start the program and close the front lid while the King Fisher is running.
12. After completion of the run, a final prompt will appear to unload the plates.
13. Press the “Check Mark”, then unload the plate containing the eluted viral RNA. Transfer the RNA
to labelled containers and store appropriately until further use.

Note

Final eluate volume varies depending on evaporation loss during warm elution step.

14. Unload the sample plate and empty contents into beaker with disinfectant. Switch on UV light for
disinfection by selecting the program in the maintenance protocols.

Changing the magnetic head on the Kingfisher Duo Prime

- 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 12/6 tip protocol with a dummy test plate containing the tip comb in the required row to ensure the right positioning. Unload the test plate.

4 Workflow B - Manual extraction

Continued from Step 2.

4.1 Bead capture and washes:

1. Add 60 µl of prepared bead mix to each sample tube containing the lysed sample solution using a new tip for every addition and rinsing it with the sample solution to prevent loss of beads.

Note

Use a new 200 µL tip for each addition to minimise bead loss and rinse the tip gently a few times to ensure full volume is transferred.

2. 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.

3. Place processing tubes on magnet and leave for at least 3 minutes to allow for bead capture to complete when beads form a pellet against the magnet.

Note

Capture times may vary. Pellets may also be smeared on the tube's wall as well as forming a compact body. Twisting the tube gently helps making the pellet more compact if needed.

4. Carefully aspirate and discard supernatant without disturbing the beads.
5. It is important to remove the lysis supernatant fully, so a brief centrifugation before collecting the remaining supernatant might be necessary.
6. Remove tube from magnetic stand and place in tube rack for washing with Wash Solution 1.
7. Add 300 µl Wash Solution 1, supplemented with Isopropanol to each sample and vortex at moderate speed for 30s.

Note

Pellets do not necessarily get fully resuspended in all samples and controls.

8. Centrifuge briefly to collect tube content.
9. Capture beads on magnet for 3-5 min or until mixture becomes clear, indicating full capture.

10. Carefully aspirate and discard supernatant.
11. Repeat steps 7-10 one more time to complete two washes with Wash Solution 1.
12. Remove tube from magnetic stand and place in tube rack for washing with Wash Solution 2.
13. Add 450 µl Wash Solution 2 supplemented with ethanol to each sample and vortex at moderate speed for 30s.

Note

Beads often appear granular during washing steps with Was Solution 2.

14. Centrifuge briefly to collect tube content.
15. Capture beads on magnet for 3-5 min or until mixture becomes clear, indicating full capture.
16. Carefully aspirate and discard supernatant.
17. Repeat steps 13-16 to complete two washes with Wash Solution 2. It is important to completely remove the supernatant after the second wash to avoid inhibition in downstream applications.

4.2 Drying the beads and elution:

18. Centrifuge briefly and remove any residual solution with fine-tipped pipette.
19. Dry the beads by leaving the tube open for 2 minutes to
20. Add 50 µL Elution Buffer to each sample and shake/vortex vigorously for 4 min.allow any remaining alcohol to evaporate.

Note

Mix until pellet resuspends fully; this usually happens without much problem.

21. Centrifuge briefly to collect tube content.
22. 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 them on the magnet to avoid tube content flipping on sidewall. When this happens, remove tube from magnet and centrifuge briefly before replacing on the magnet.

Note

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

Note

Pellet might be smeared around the bottom of the tube as opposed to forming a compact pellet against the magnet. Look into the open tube from above to check if eluate is clear. Aim to collect RNA from a central position without touching the pellet.