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Large volume viral RNA extraction using MagMAX Viral RNA Isolation Kit V.3

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Poliovirus Sequencing C...

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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.

Start by preparing the reagents required for the number of samples extracted, including the controls. To perform manual extractions follow steps in Workflow A, for automated extraction using KingFisher Duo Prime device follow Workflow B and for automated extraction on King Fisher Flex machine follow Workflow C. Workflows diverge after Step 2, the initial sample processing with Lysis/Binding solution.

KingFisher programs are attached for installation.

Protocol version 2 is slightly edited and expanded to include workflow for KingFisher Flex, updated plate maps and listed KingFisher plasticware in the materials section.

Protocol version 3 has typo corrections and slight editing in King Fisher Flex protocol steps.

Materials

Equipment

- Biological Safety Cabinet II (Class II BSC), PPE
- Magnetic stand(s) accommodating 5/15 mL centrifuge tubes / King Fisher Duo Prime equipped with 6 tip-magnetic head / King Fisher Flex equipped with 24-tip magnetic head
- 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 mL /15 mL tubes
- Serial Dispenser pipette with appropriate tips is recommended for use to avoid repetitive strain injury and maintained accuracy, but it is not essential

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.

Plasticware for King Fisher Duo Prime extraction:

	A	B
	Item	**ThermoFisher product code**
	KingFisher Duo Prime 6-tip comb and 24 deep-well plate (12 pieces of 24 deep-well plates, each containing 4 tip combs)	97003510
	King Fisher 24 deep-well plate (50)	95040470

Plasticware for King Fisher Flex extraction:

	A	B
	Item	**ThermoFisher product code**
	KingFisher 24 deep-well plate (50)	95040470
	KingFisher 24 deep-well tip comb and plate (50)	97002610

Reagent Preparation

1 Clean reagents should be prepared in a separate clean area away from the samples.

Carefully consider the number of samples to be processed, depending on the extraction workflow used.

Wash Solutions only need to be prepared once and can be used until exhausted.

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.
- Mark the bottle and store at temperature shown on the label.

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.
- Mark the bottle and store at temperature shown on label.

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

1.3 Lysis/Binding Solution

Prepare enough solution for the number of samples extracted that day considering the practical capacity of the used workflow and including controls. Allow extra for pipetting loss.

Combine the components listed below in the order indicated.

- 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**

	A	B
	Nucleic Acid Binding Beads	30 µl
	Lysis ENHANCER/ Proteinase K	30 µl
	Total volume	60 µl

Bead mix preparation

- Mix well by vortexing and store appropriately until needed.

Sample Preparation

2 **Sample Processing**

Perform sample lysis in a dedicated sample handling work area away from where only clean reagents are handled.

Due to their ease of use and specific surface treatment, the 5 mL Eppendorf DNA LoBind microcentrifuge tubes are recommended for use. In case these are not available, regular sterile 15 mL conical bottom centrifuge tubes can be used instead as they have the same diameter and fit the same centrifuge rotors and magnetic racks.

2.1 Prepare the lysate in 5 mL or 15 mL 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 sewage concentrate samples to the tubes containing the Lysis/Binding solution.

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Note

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.

Manual Extraction - Workflow A

3 Continued from Step 2.

The number of samples to be processed simultaneously is determined by the capacity of the magnetic rack(s) used.

3.1 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.

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.

3.2 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.

Automated extraction using King Fisher Duo Prime - Worklfow B

4 Continued from Step 2.

MVRI_DUO_LV_1200ul.bdz 3.7KB

Read the King Fisher Duo Prime instrument manual for installation and operating instructions in its entirety before operating the magnetic particle processor. Download and import the attached *MVRI_Duo_LV_1200ul* program onto your device and perform a mock run.

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

	A	B
	Item	**ThermoFisher product code**
	KingFisher Duo Prime 6-tip comb and 24 deep-well plate (12 pieces of 24 deep-well plates, each containing 4 tip combs)	97003510
	King Fisher 24 deep-well plate	95040470

Plasticware for large volume extractions on King Fisher Duo Prime

On the King Fisher Duo Prime device up to 6 samples can be extracted at the same time using 24 deep well plates and 6-tip combs.

4.1 Preparing the 24 deep well plates

Note

Prepare plates in the clean reagent handling area. Move them to sample handling area only when ready to add the processed samples.

1. Label two 24 deep-well plates as Sample plate and Elution plate. Prepare and clearly label more plates of each if the number of samples requires.

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

	Plate position	Plate type	Reagent	Volume per well
Sample lysis and binding	Sample plate, Row A	King Fisher 24 deep well plate	Lysis/Binding solution+cRNA+100%Isopropanol	2.4 mL
			Sample	1.2 mL
			Bead mix	60 µL
First Wash 1	Sample plate, Row B	King Fisher 24 deep well plate	Wash Solution 1	900 µL
Second Wash 1	Sample plate, Row C	King Fisher 24 deep well plate	Wash Solution 1	900 µL
6-Tip comb	Sample plate, Row D	MME-24 deep 6 well Tip comb		
First Wash 2	Elution plate, Row A	King Fisher 24 deep well plate	Wash Solution 2	1350 µL
Second Wash 2	Elution plate, Row B	King Fisher 24 deep well plate	Wash Solution 2	1350 µL
Elution	Elution Plate Row D	King Fisher 24 deep well plate	Elution Buffer / nuclease free water	90 µL

2. Add 900 µl of prepared Wash Solution 1 to rows B and C of the Sample Plate.
3. Place a 12-tip comb in a 96 deep well plate in row D of the Sample Plate.
4. Add 1350 µl of prepared Wash Solution 2 to rows A and B of the Elution Plate
5. Add 90 µl of Elution Buffer or nuclease free water to row D in the Elution Plate.
6. Move the prepared plates to the sample handling area. Transfer the 3.6 ml of sample/lysis mixture to row A of plate 1.
7. 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.

4.2 Setting up and running the King Fisher Duo Prime

Check to confirm that the King Fisher Duo Prime is set up with 6 tip magnet and heating block. Change the magnetic head and heating block if necessary.

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

8. Select the program MVRI Duo LV 1200ul and load the two plates using the A1 markings as prompted. Ensure that the plates are lying completely flat. The two plates will be on opposing sides of the turning platform in the processor.
9. Start the program and close the front lid while the King Fisher is running.
10. After completion of the run, a final prompt will appear to unload the plates.
11. 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 warming steps.

12. 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.

Automated extraction using King Fisher Flex - Workflow C

5 Continued from Step 2.

MVRI_Flex_LV_1200ul.bdz 3.8KB

Read the King Fisher Flex instrument manual for installation and operating instructions in its entirety before operating the magnetic particle processor. Download and import the attached *MVRI_Flex_LV_1200ul* program onto your device and perform a mock run.

Plasticware for large volume sample extractions on King Fisher Flex equipped with 24-tip magnetic head.

	A	B
	Item	**ThermoFisher product code**
	KingFisher 24 deep-well plate (50)	95040470
	KingFisher 24 deep-well tip comb and plate (50)	97002610

Plasticware for King Fisher Flex 24 deep-well plate extraction

On the King Fisher Flex device up to 24 samples can be extracted at the same time using 24 deep well plates and 24-tip combs.

5.1 Setting up the processing plates:

Note

Prepare plates in the clean reagent handling area. Move the Sample plate to the sample handling area only when ready to add the processed samples.

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

Plate	Plasticware	Reagent	Volume per well
Sample lysis and binding	King Fisher 24 deep well plate	Lysis/Binding solution+cRNA+100%Isopropanol	2.4 mL
		Sample	1.2 mL
		Bead mix	60 µL
First Wash 1 (FW1)	King Fisher 24 deep well plate	Wash Solution 1	900 µL
Second Wash 1 (SW1)	King Fisher 24 deep well plate	Wash Solution 1	900 µL
Tip comb plate	King Fisher 24 deep-well tip comb		
First Wash 2 (FW2)	King Fisher 24 deep well plate	Wash Solution 2	1350 µL
Second Wash 2 (SW2)	King Fisher 24 deep well plate	Wash Solution 2	1350 µL
Elution	King Fisher 24 deep well plate	Elution Buffer / nuclease free water	90 µL

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.

2. Add 900 µl of prepared Wash Solution 1 plates FW1 and SW1.
3. Add 1350 µl of prepared Wash Solution 2 to plates FW2 and SW2.
4. Place a 24-tip comb in Tip comb Plate.5.
5. Add 1350 µl of prepared Wash Soution 2 to Plate Second Wash II.
6. Add 90 µl of Elution Buffer or nuclease free water the Elution Plate.
7. Label a Sample plate and take it to the sample handling area to add the processed samples from
Step 2.

5.2 Preparing the Sample plate and running the extraction program:

8. Transfer the 3.6 ml of sample/lysis mixture from each sample processing tube to the labelled Sample plate.
9. Add 60 µl of prepared Bead Mix to wells in the 24 deep well 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.
10. Check to confirm that the instrument is set up with 24 deep-well magnetic head and 24 deep-well heat block. Select the MVRI_Flex_LV_1200ul protocol on the equipment and load the plates onto the King Fisher Flex as directed, then start the protocol.

11. Close the front lid of the device.

5.3 Unloading the device:

12. After completion of the run, a final prompt will appear. “Unload elution plate”.
13. Transfer eluted viral RNA to labelled containers and keep on ice for immediate use or store frozen until needed.

Note

Final eluate volume varies depending on evaporation loss during warming steps.

14. Remove plates as prompted and wipe equipment clean as necessary. Be careful not to apply pressure to the turntable. Dispose of processing plates and their contents by following standard laboratory processes.