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Purification of viral RNA from cell culture isolates or FTA cards using MagMAX Viral RNA Isolation Kit

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ABSTRACT

This protocol describes the viral nucleic acid recovery and purification from poliovirus isolates either in their original liquid form or spotted onto FTA cards. The method utilises the magnetic bead based MagMAX ™ Viral RNA Isolation Kit. When using isolates directly, progress to one of the extraction workflows straight after reagent preparation, skipping Section 2. To process FTA card discs follow the procedures in Sections 1 and 2 in full before extraction. 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.

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We use this protocol and it's working

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MATERIALS

Reagents

- Ethanol (96-100%)
- Isopropanol (100%)
- TE buffer (10mM TRIS-HCl/0.1mM EDTA pH 8) (Thermo Fisher, Cat. No. 12090-015)
- Glycogen, Nuclease Free (Ambion Cat. No. AM9510)
- DTT (Dithiothreitol powder, Sigma-Aldrich Cat. No. D-9779 or 1M solution in ready to use, sterile formulation)
- Nuclease free water (for preparing 1M DTT solution)
- 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

	A	B	C	D
	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

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
- RNaseZap RNase Decontamination Solution (Thermo Fisher, Cat. No. AM9780)
- Themomixer or heating block accommodating 1.5 mL/ 2mL microcentrifuge tubes incubating at 70 °C
- Vortex/shaker/rotator/thermomixer accommodating 1.5-2 mL centrifuge tubes
- 4mm Disposable Biopsy Punch with plunger (Miltex, Northumbrian Medical Supplies, Cat. No.
  33-34-P/25)
- Cutting Mat (GE Healthcare, Cat.No. WB100020)
- Sharps Waste container to dispose used biopsy punchers
- 0.45µm syringe filter with sterile 5 mL syringe for sterile filtration of 1M DTT if prepared from powder
- Centrifuge accommodating 1.5-2 mL tubes

- Plasticware for the use of King Fisher Duo Prime equipment with 12-tip magnet, as shown in Workflow B
- Plasticware for the use of King Fisher Flex equipment with 96-tip magnetic head, as shown in Workflow C

Reagent Preparation

15m

1 To process samples from FTA cards, prepare all the reagents listed, to start from liquid cell culture isolates prepare reagents in steps 1.3-1.6 only.

1.1 DTT solution (1M) 5m

- Dissolve 0.6168g of DTT in 4 ml of Nuclease Free water.
- Sterilize by filtration with 0.45 µm syringe filter unit and a 5 ml syringe.
- Prepare aliquots and store at -20°C.

1.2 RNA Processing Buffer 3m

Combine the components below to prepare enough RNA Processing Buffer for the required number of samples and store on ice. RNA Processing Buffer can also be made ahead of time and stored at -20 °C
for up to six months.

1.3 Wash Solution 1 1m

- 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.

1.4 Wash Solution 2 1m

- 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 contamination if they are to be used on multiple occasions.

1.5 Lysis/Binding Solution

3m

Defrost Carrier RNA on ice and 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.

- 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	200 µl
Carrier RNA	1 µl

- Add 100% isopropanol and mix well by vortexing.

A	B
Component	**Per sample**
100% Isopropanol	200 µl

Note

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

1.6 Bead Mix

2m

- Vortex the nucleic acid binding beads well to ensure that the beads are fully resuspended.
- Combine the components that are listed below:

A	B
Component	**Volume per sample**
Nucleic Acid Binding Beads	7 µl
Lysis/Binding ENHANCER	7 µl
Total volume	**14 µl**

- 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.

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.

FTA card Processing

22m

2 Follow the below steps if using FTA cards. For liquid isolates, progress to RNA extraction section following workflow A/B/C depending on the mode of extraction.

2.1 Set the thermal block to 70°C.

2.2 For each sample:
Set up and label clean nuclease free 1.5 mL microcentrifuge tubes for each FTA card to be processed.

2m

2.3 Open one FTA card pouch and take out the card. Place the cutting mat under the FTA card marked area, where the paper will be punched.

2.4 Using a new 4mm disposable punch, punch out seven (7) punches per sample and place in the labelled 1.5 ml microtube. (Do not punch too hard or the punch will dull and not cut). 5m

Note

Avoid moving the FTA card around on the cutting mat, so as not to contaminate the cutting surface.

Note

Ensure FTA card is punched in a manner to allow for additional round of extraction if necessary with positioning the punched holes accordingly.

2.5 Dispose of the biopsy punch in the sharps bin and use new punch for each card.

2.6 Place the next card in a different spot on the cutting mat. The front and back of the mat may be used before having to clean the mat. (Do not use the same spot as before to punch a new card to avoid contamination between cards).

2.7 Aliquot 300 µl of RNA Processing Buffer into each tube with the FTA card discs. 2m

2.8 Close and vortex tubes at high speed for 40 seconds. 5m

2.9 Incubate tubes at 70°C for 5 minutes at 800 rpm speed. (If not using thermomixer, take tubes and mix by vortexing briefly at moderate speed every 2 minutes to a total of 3 times.) 6m

Note

Ensure the tube lids are properly closed during incubation to prevent popping open during mixing.

2.10 When incubation is over, centrifuge tubes briefly to collect content. (You will be able to recover u 2m to ~240 µl of supernatant for RNA extraction from each sample).

Process sample supernatants to extract viral RNA by following one of the extraction workflows in step 3/4/5.

RNA extraction 7h 8m

3 Manual extraction – Workflow A 1h 10m

The indicated processing times are based on handling 12 samples at the same time .

3.1 Prepare the lysate: 10m

For each sample:

1. Set up and label 1.5 ml Eppendorf DNA LoBind centrifuge tubes, then aliquot 400 µl of the lysis/binding solution (supplemented with carrier RNA and 100% Isopropanol - see Reagent preparation section) into each tube.

2. Transfer 200 µl of the liquid isolate or the full recoverable volume from the processed FTA card 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.

3.2 Bead capture and washes: 50m

4. Add 14 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 200 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 300 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.

3.3 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 efficiency 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.

4 Automated extraction using King Fisher Duo Prime – Workflow B

54m

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

Continued from Step 2.

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
	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

4.1 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	400
			clarified sample	200-240
			Bead mix	14
First wash 1	B	MME -96 deep well plate	Wash solution 1	200
Second wash 1	C	MME -96 deep well plate	Wash solution 1	200
First wash 2	D	MME -96 deep well plate	Wash solution 2	300
Second wash 2	E	MME -96 deep well plate	Wash solution 2	300
	F			
	G			
12-Tip comb	H	MME-96 deep well plate	MME-96 deep well tip comb in standard plate	
Elution		Elution strip tube	60 µl Elution buffer / nuclease free water	

1. Aliquot 400 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 200 µl of the liquid isolate or all the recovered volume (200-240 µl) of the processed FTA card supernatant 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 14 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 200 µl of prepared wash solution 1 to rows B and C of the 96 deep well plate.

6. Add 300 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 60 µl of Elution buffer/ nuclease free water to the elution strip tube.

4.2 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.

4.3 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.

5 Automated extraction using King Fisher Flex – Workflow C

1h 30m

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

Continued from step 2.

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

5.1 Preparing sample plate

30m

1. Aliquot 400 µl of the Lysis/Binding solution supplemented with carrier RNA and 100% Isopropanol (see step 9 and 10 in Reagent preparation) to a 96 deep-well plate marked as Sample plate.

2. Add the recovered volume (200-240 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.

5.2 Setting up the processing plates and running the extraction program

55m

3. Add 14 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+ 10% Isopropanol	400
		clarified sample	200-240
		Bead mix	14
First wash 1 (FW1)	96 deep well plate	Wash solution 1	200
Second wash 1 (SW1)	96 deep well plate	Wash solution 1	200
First wash 2 (FW2)	96 deep well plate	Wash solution 2	300
Second wash 2 (SW2)	96 deep well plate	Wash solution 2	300
Elution	96 well standard plate	Elution buffer / nuclease free water	60
Tip comb plate	96 well standard plate	Tip comb in standard plate	

5. Add 200 µl of prepared wash solution 1 to the 96 deep well plates marked FW1 and SW1.

6. Add 300 µl of prepared wash solution 2 of the 96 deep well plates marked FW2 and SW2.

7. Add 60 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.

5.3 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.