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Protocol for Extraction of Viral RNA with added DNase treatment

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1 Works for me dx.doi.org/10.17504/protocols.io.bnkhmct6

Coronavirus Method Development Community

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ABSTRACT

This protocol is modified from the MagMAX MVP II protocol from Thermo Fisher, and the MagMAX mirVana protocol from Thermo Fisher. Modifications were necessary to include a DNAse treatment step, which improves viral recovery in low-burden samples.

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ABSTRACT

This protocol is modified from the MagMAX MVP II protocol from Thermo Fisher, and the MagMAX mirVana protocol from Thermo Fisher. Modifications were necessary to include a DNAse treatment step, which improves viral recovery in low-burden samples.

RNA extraction with MVP II kit and Kingrisher

1 Gather all plastic ware needed for the Kingfisher program. This will include 6 deep well plates, one tip comb, and one standard 200ul plate. Some plates can be prepared in advance to save time the day of extraction. See step 8 for more details.

KingFisher™ Flex™ Systems Consumables, KingFisher Flex Microtiter Deepwell 96 plate, V-bottom **Thermo Fisher Catalog #95040450**

KingFisher™ Systems Consumables, KingFisher tip comb **Thermo Fisher Catalog #97002070**

KingFisher™ Flex™ Systems Consumables, KingFisher 96 KF microplate (200µL) **Thermo Fisher Catalog #97002540**

RNA extraction using MVP II kit and Kingfisher

2 Aliquot 5 ul of proteinase k to each sample well of a 96-well deep-well plate to be used for extraction.

3 Add 50ul sample to wells

This can be increased to as much as 200ul if sufficient sample volume is available

4 Bring sample volume up to 200ul with 150ul molecular biology grade H20

If you increased sample volume in step 3, decrease H20 here for a final volume of 200ul.

5 Prepare Binding Bead Mix according to the following:

Component	Volume/well (ul)
Binding Solution	265
Magnetic Beads	10
Total Volume	275

Binding solution is very viscous. Pipette slowly. Make enough binding solution to allow for ~15-20% overage to account for volume lost in pipetting.

6 Add 275ul Binding Bead mix to each sample well and pipette to mix.

Mix slowly! Binding solution can become very frothy, and well-to-well contamination is possible if care is not taken at this step.

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7 Prepare Dnase solution on ice as follows. Keep on ice until needed (step 10).

Component	Volume/well (ul)
Turbo Dnase buffer	48
Turbo Dnase	2
Total Volume	50

TURBO™ DNase (2 U/µL) **Thermo Fisher Scientific Catalog #AM2238**

8 Prepare remaining reagents as follows:

Plate ID	Plate type	Reagent	Volume/well (ul)
Wash 1	Deep Well	Wash Solution	1000
Wash 2	Deep Well	80% Ethanol	1000
Dnase	Deep Well	Dnase solution	50
Wash 3	Deep Well	80% Ethanol	500
Wash 4	Deep Well	80% Ethanol	500
Elution	Standard 200ul	Elution Solution	50
Tip Comb	Deep Well	none	none

Wash and elution plates can be prepared ahead of time. Be sure to seal plates tightly to avoid evaporation, and centrifuge briefly prior to use.

9 Run the following program on the Kingfisher flex. Add the plates from steps 1-8 to the machine when prompted to do so.

MVPII_withdnase.bdz

Kingfisher Flex
Automated Extraction System
ThermoFisher **5400630**

10 When prompted, ~30minutes into protocol, remove DNAse plate from Kingfisher and add 50ul binding solution and 150ul isopropanol (molecular biology grade) to each sample well

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11 Return Dnase plate to Kingisher and press start to continue protocol.

12 When run has finished, remove elution plate, seal, and store at -80C.

13 Discard remaing plates into appropriate biohazard waste container.

Optional QC

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Optional QC

Run a sub-sample of extracted RNA on a Bioanalyzer or Tape Station to check RNA quality and DNA removal success.

Bioanalyzer 6000 Pico RNA kit **Agilent Technologies Catalog #5067-1513**

Bioanalyzer 6000 RNA Pico Ladder **Agilent Technologies Catalog #5067-1535**

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