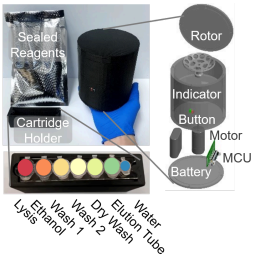
Oct 28, 2024

A Portable Device for Lab-Free, Versatile Nucleic Acid Extraction - Protocol



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Protocol status: Working
As of Oct. 2024, this protocol has been tested and validated for use with viral RNA from plasma or saliva, as well as for miRNA from saliva.

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Abstract

To enable faster, easier extraction of viral RNA outside of traditional laboratories, we use a custom, automated, and portable centrifuge for processing Qiagen QIAamp RNA extractions.

Viral RNA is eluted into approx. 60 µl of nuclease free water that can then be analyzed by downstream testing (PCR, gel electro, Qubit). Our results showed very strong agreement with benchtop extractions and probit analysis demonstrated a limiting extraction concentration of 0.75 cp/µl at 95% confidence.

Guidelines

This has now been tested on a variety of patient samples including HIV in plasma, SARS-CoV-2 in saliva, SARS-CoV-2 in VTM (from nasopharygeal swab), and miRNA in saliva (as of October 15,2024).

Materials

RNA EXTRACTION

- Qiagen - QIAamp Viral RNA Mini Kit

PRIMERS

- Primers were purchased from IDT.

RT-PCR

- Applied Biosystems - Fast Virus 1-Step Master Mix

Protocol materials

Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix for qPCR **Applied Biosystems (ThermoFisher Scientific)** **Catalog #**44-444-32

QIAamp Viral RNA Mini Kit **Qiagen** **Catalog #**52904

Safety warnings

Please follow standard health and safety guidelines when working with viral samples.

Sample Collection

1 Label a 1.5 mL tube and add each 50 µl Sample .

> Note
>
> The following reagent volumes and steps are assuming a 50 µl starting sample.

> Note
>
> If you need to process more than 50 µl, increase the lysis buffer and ethanol proportionally to your new sample volume (550 µl : 50 µl).
> A maximum of 750 µl will fit into the top of the mini-column, therefore if you increase sample volume you will need to add additional repetitions of the RNA/DNA binding steps.
> **Please see the manufacturer's manual for additional details.**

2 Gather QIAamp Viral RNA Mini Kit **Qiagen Catalog #**52904

RNA/DNA Lysis

3 Add 550 µL Lysis buffer to the 50 µl Sample 10m

Mix well by vortexing or pipetting.

Incubate for 00:10:00 at Room temperature

> Expected result
>
> 600 µL DNA Lysate

Power On Portable Centrifuge

4 Turn on the power switch located on the rear of the device.

5

Expected result

A bright red LED should illuminate on the front of the device, indicating the device is ready to be used.

Note

Our device is pre-programmed to manage the spin down steps and automatically adjust the timings depending on the appropriate step. Example: Step 1. 60s Step 2. 60s etc.

At this time the use of other devices is not recommended or tested with this protocol. We imagine mini-centrifuges operating on external battery power could work as an alternative if they maintain higher than 1750g while spinning. (Can check with equation listed below)

$$RPM = \sqrt{\frac{RCF}{1.118 \times 10^{-5} \times r}}$$

RNA/DNA Binding

6 Add 550 µL Ethanol (95%) to the 600 µL DNA Lysate

Expected result

1150 µL DNA Lysate

7 Mix well and pipette 750 µL DNA Lysate / 1150µl into the top of the QIAamp Mini Spin Column.

8 Place the entire QIAamp Mini Spin Column & tube into the portable centrifuge.

9 Close the lid of the device, check the red LED is illuminated, and push the front button beside the LED.

> Note
>
> If the red LED is not illuminated check that Step 4 was completed, and that the device was charged overnight.

10 Wait 00:01:00 for the liquid to spin down. 1m

11 Remove the QIAamp Mini Spin Column & discard the waste tube.

12 Insert the QIAamp Mini Spin Column into a fresh collection tube.

13 Repeat Steps 7 → 12.

RNA/DNA Wash 1

14 Add 500 µL Buffer AW1 to the top of QIAamp Mini Spin Column.

15 Place the entire QIAamp Mini Spin Column & tube into the portable centrifuge.

16 Close the lid of the device, check the red LED is illuminated, and push the front button beside the LED.

17 Wait 00:01:00 for the liquid to spin down.

18 Remove the QIAamp Mini Spin Column & discard the waste tube.

19 Insert the QIAamp Mini Spin Column into a fresh collection tube.

RNA/DNA Wash 2

20 Add 500 µL Buffer AW2 to the top of QIAamp Mini Spin Column.

21 Place the entire QIAamp Mini Spin Column & tube into the portable centrifuge.

22 Close the lid of the device, check the red LED is illuminated, and push the front button beside the LED.

23 Wait 00:01:00 for the liquid to spin down.

24 Remove the QIAamp Mini Spin Column & discard the waste tube.

25 Insert the QIAamp Mini Spin Column into a fresh collection tube.

RNA/DNA Drying

26 Add 500 µL Ethanol (95%) to the top of QIAamp Mini Spin Column.

27 Place the entire QIAamp Mini Spin Column & tube into the portable centrifuge.

28 Close the lid of the device, check the red LED is illuminated, and push the front button beside the LED.

29 Wait 00:03:00 for the liquid to spin down. 3m

30 Remove the QIAamp Mini Spin Column & discard the waste tube.

31 Insert the Mini Column into a fresh 1.5 mL tube and label accordingly.

RNA/DNA Elution

32 Add 80 µL Nuclease Free Water to the top of QIAamp Mini Spin Column. Be sure to drop liquid into the center of the column membrane.

> Note
>
> In this protocol we used Nuclease Free Water to elute our samples for highest compatibility with downstream PCR. For other applications replace with Buffer AVE.

> Note
>
> In this protocol we used 80 µl Nuclease Free Water to elute our samples. For other applications this volume could be adjusted. For highest yield a two-stage elution with 1/2 and 2/2 elution volume can be used, but was not tested in this protocol. Please see the Qiagen Viral RNA Mini Kit manual for more details when designing for your specific application.

33 Incubate 00:01:00 at Room temperature 1m

34 Place the entire QIAamp Mini Spin Column & tube into the portable centrifuge.

35 Close the lid of the device, check the red LED is illuminated, and push the front button beside the LED.

36 Wait 00:01:00 for the liquid to spin down.

37 Collect the labeled 1.5 mL tube.

Expected result

The 1.5 mL tube now contains ~50-60 µl of RNA sample in Nuclease Free Water. Please store appropriately (or move immediately to downstream testing.

38 Discard the QIAamp Mini Spin Column.

RT-PCR

5m 53s

39 Thaw at Room temperature and place on ice:

1. PCR Master Mix
2. PCR primers
3. Water
4. RNA Sample

The PCR Mix used in this protocol:

Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix for qPCR **Applied Biosystems (ThermoFisher Scientific) Catalog #**44-444-32

Primers were purchased from Integrated DNA Technologies (IDT).

40 Setup the following reaction:

Component (Stock Concentration)	Volume
PCR Master Mix (4X)	6.25 µL
Forward primer (10 µM)	1.50 µL
Reverse primer (10 µM)	1.50 µL
RNA Sample (variable)	10 µL
Nuclease Free Water	5.50 µL
Total	25 µL

41 Set-up the following program on the thermal cycler: 5m 53s

Step	Temperature	Time	Cycles
Reverse Transcription	55 °C	00:05:00	1
Heat Activation	95 °C	00:00:20	1
Denaturation	95 °C	00:00:03	45-60
Annealing & Extension	60 °C	00:00:30	45-60
Hold	4 °C		

Protocol references

https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits

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