VERSION 2

JAN 05, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bpg5mjy6

Protocol Citation: Adélaïde Roguet, Shuchen Feng 2021. Nucleic acid extraction - RNeasy PowerMicrobiome Kit (Qiagen). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bpg5mjy6> Version created by [McLellan Lab](#)

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Protocol status: In development
 We are still developing and optimizing this protocol

Created: Nov 07, 2020

Last Modified: Jan 05, 2021

PROTOCOL integer ID:
 44285

Nucleic acid extraction - RNeasy PowerMicrobiome Kit (Qiagen) V.2

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Coronavirus Method Development Community

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GUIDELINES

When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 10% bleach, let stand for 10 min, rinse with water, then with 70% ethanol, and finally with RNAase AWAY.

MATERIALS

MATERIALS

RNeasy PowerMicrobiome Kit **Qiagen Catalog #26000-50**

Equipment	
Mini-Beadbeater-16	NAME
high-energy cell disrupter	TYPE
BioSpec	BRAND
607	SKU
https://biospec.com/product/mini-beadbeater-16	LINK
1 speed	SPECIFICATIONS

- β -mercaptoethanol
- 0.5 mL free-standing microcentrifuge tubes (low binding)

SAFETY WARNINGS

- ! The nucleic acid extraction has to be performed in a chemical safety to avoid any inhalation of beta-mercaptoethanol.

BEFORE START INSTRUCTIONS

1. Clean the working area and all equipment: wipe down with 70% ethanol and let dry. Then, wipe down using RNase AWAY and let dry.
2. Warm RNase-Free Water at 55C.

Nucleic acid extraction (in the chemical safety cabinet)

7m

- 1 For **HA filter** extraction, add 6.5 µL of beta-Mercaptoethanol to each of the 2-mL tubes. Go to **step 2**.

For **BCoV** extraction, add 5 µL of beta-Mercaptoethanol to each of the 2-mL tubes. Warm the tube at 55C for 5 min and vortex for 15 seconds. Let sit for 10 min. Skip step 2.

Safety information

Add the beta-Mercaptoethanol while samples are still frozen. Let thaw at room temperature.

2

For **HA filter** extraction, place the 2-mL tubes in the bead beater.

Equipment

Mini-Beadbeater-16

NAME

high-energy cell disrupter

TYPE

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607

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LINK

1 speed

SPECIFICATIONS

2.1 Bead beat for 00:02:30

2m

Safety information

Start the bead beating when the beads start to be loose in the tubes.

2.2 Cooldown the samples on ice for 00:05:00 .

5m

2.3 Repeat Steps 9.1 and 9.2 once .

3 Centrifuge at maximum speed for 1 min at room temperature.

150000 rpm, Room temperature, 00:01:00

4 For **HA filter** extraction, transfer 450 μ L of supernatant to a Collection Tube (provided in the RNeasy PowerMicrobiome kit).
For **BCoV** extraction, transfer all supernatant.

- 5 Add 150 μ L of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
- 6 Centrifuge at maximum speed for 1 min 150000 rpm, Room temperature, 00:01:00 .
Avoiding the pellet and transfer the supernatant to a new Collection Tube.
- 7 Add 650 μ L each of Solution PM3 and Solution PM4. Vortex briefly to mix.
- 8 Load 650 μ L of the mixture into an MB Spin Column.
- 9 Centrifuge at max speed for 1 min 150000 rpm, Room temperature, 00:01:00 . Discard the flow-through and repeat until all the mixture has been loaded onto the MB Spin Column.
- 10 Shake to mix Solution PM5 and add 650 μ L to the MB Spin Column.
- 11 Centrifuge at max speed for 1 min 150000 rpm, Room temperature, 00:01:00 .
- 12 Discard flow-through. Add 600 μ L of Solution PM4.

- 13 Centrifuge at max speed for 1 min 150000 rpm, Room temperature, 00:01:00 .
- 14 Discard flow-through and centrifuge filter at max speed for an additional 2 min 150000 rpm, Room temperature, 00:02:00 .
- 15 Place the MB Spin Column in a clean 2-ml Collection Tube (provided in the RNeasy PowerMicrobiome kit).
- 16 Add 60 μ L of RNase-Free Water (warmed to 55°C) to the center of the MB Spin Column membrane. Incubate at room temperature for at least 1 min 00:01:00 .
- 17 Centrifuge at max speed for 1 min 150000 rpm, Room temperature, 00:01:00 . Discard the MB Spin Column.

Safety information

The DNA/RNA is now ready for downstream applications. RNA extract may be stored in RNase-free water at -80°C for 1 year.