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Nucleic acid extraction - RNeasy PowerMicrobiome Kit (Qiagen) V.3

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ABSTRACT

Total nucleic extraction from wastewater using RNeasy PowerMicrobiome kit (qiagen)

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PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

Prepare bovine coronavirus (BCoV) solution

Concentration of viruses from sewage using HA filters

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 TEXT

MATERIALS

RNeasy PowerMicrobiome

Kit **Qiagen Catalog #26000-50**

Mini-Beadbeater-16
high-energy cell disrupter
BioSpec **607**
1 speed

- 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.

DISCLAIMER:

BEFORE STARTING

1. Clean the working area and all equipment: wipe down with 10% bleach and let dry. Wipe down with 70% ethanol and let dry. Then, wipe down using RNase AWAY and let dry.
2. Warm RNase-Free Water at 55C.

For **HA filter** extraction: put on ice the 2-mL ZR BashingBead Lysis tubes containing 1x Filter + 650 µL of PM1.
For **BCoV/BRSV** extraction: in each 2-mL tube, add 500 µL of warmed PM1 (55C) + 5 µL beta-mercaptoethanol.
For **Direct extraction**: in each 2-mL tube, add 400 µL of warmed PM1 (55C) + 4 µL beta-mercaptoethanol.

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 ZR BashingBead Lysis tubes. Go to **step 2**.

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

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For **BCoV/BRSV** extraction, add 5 µL of BCoV/BRSV solution to the 2-mL tube containing the warmed PM1 + beta-Mercaptoethanol solution. Vortex for 15 seconds (speed 7 out of 10) and let sit for 10 min. Skip step 2.

For **Direct extraction**, add 150 µL of wastewater to the 2-mL tube containing the warmed PM1 + beta-Mercaptoethanol solution. Vortex for 15 seconds (speed 7 out of 10) and let sit for 10 min. Skip step 2.

2

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

Mini-Beadbeater-16
high-energy cell disrupter
BioSpec **607**
1 speed

2m

2.1 Bead beat for **00:02:30**

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

5m

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

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 µL of supernatant to a Collection Tube (provided in the RNeasy PowerMicrobiome kit).
For **BCoV/BRSV/Direct extraction**, transfer all supernatant.

5 For **HA filter/BCoV/BRSV** extraction, add 150 µL of Solution IRS.
For **Direct extraction**, add 100 µL of Solution IRS.

5m

6 Vortex briefly to mix (speed 7 out of 10). Incubate at 2–8°C for 5 min. **00:05:00**

Place the tubes in a cold rack (stays in the refrigerator).

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7 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.

8 Add 650 µL each of Solution PM3 and Solution PM4. Vortex by inverting the tubes 15 times.

Visually, check that the 3 solutions are well mixed.

9 Load the mixture into an MB Spin Column.

9.1 **Using centrifuge:** 1m

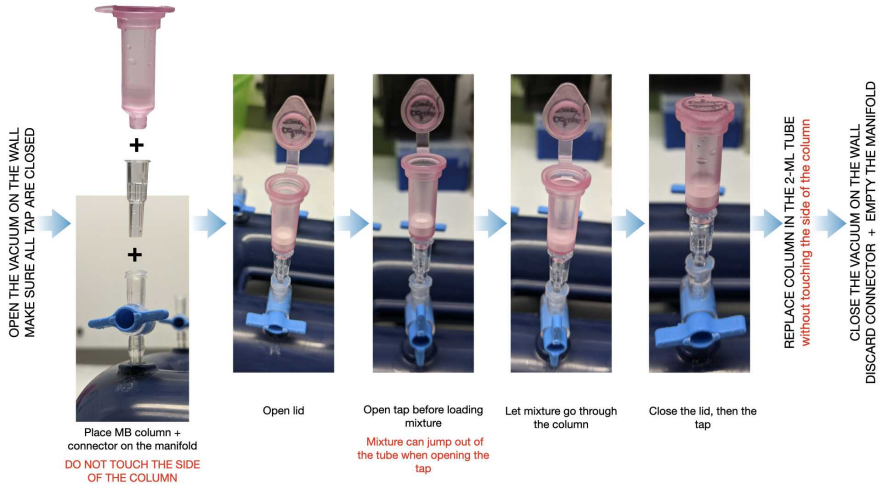
- Load 650 µL of the mixture into an MB Spin Column

- 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.

9.2 **Using manifold:** 1m

- Load the all mixture into an MB Spin Column (see picture)

- Centrifuge at max speed for 1 min **150000 rpm, Room temperature , 00:01:00** . Discard the flow-through.



10 Shake well to mix Solution PM5 and add 650 µL to the MB Spin Column.

11 Centrifuge at max speed for 1 min **150000 rpm, Room temperature , 00:01:00** .

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12 Discard flow-through. Add 600 µL 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 µL of RNase-Free Water (warmed to 55ºC) to the center of the MB Spin Column membrane. Incubate at room temperature for 5 min **00:05:00** . 5m

17 Centrifuge at max speed for 1 min **150000 rpm, Room temperature , 00:01:00** . Discard the MB Spin Column.

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

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