Nucleic acid extraction - RNeasy PowerMicrobiome Kit (Qiagen) V.1

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PARENT PROTOCOLS

In steps of

Prepare bovine coronavirus (BCoV) solution
Concentrate viruses from sewage using HA filters
Concentration of viruses from sewage using HA filters
Prepare bovine coronavirus (BCoV) solution

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.

BEFORE STARTING

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** extractions, Add 6.5 µL of beta-Mercaptoethanol to each of the 2-mL tubes.

For **direct extraction** of raw sewage, homogenize the sewage sample (avoid foaming) and take 150 µL into a 2-mL tube (provided in the RNeasy PowerMicrobiome kit). Add 4 µL of beta-Mercaptoethanol and 400 µL of Solution PM1. Also add 5 µL of 1:10 diluted BCoV solution.

Add the beta-Mercaptoethanol while the HA sample still frozen. Let thaw at room temperature.

2

If you extract from **HA filters**, place the 2-mL tubes in the bead beater.
Skip step 2 if you do **direct extraction**.

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

2.1 Bead beat for **00:02:30** 2m

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 To extract from **HA filters**, transfer 450 µL of supernatant to a Collection Tube (provided in the RNeasy PowerMicrobiome kit). Transfer all supernatant for **direct extraction**.

5 To extract from **HA filters**, add 150 µL of Solution IRS and vortex briefly to mix. For **direct extraction**, add 100 uL of Solution IRS. 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 µL each of Solution PM3 and Solution PM4. Vortex briefly to mix.

8 Load 650 µL 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 µL 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 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 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.

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