

May 02, 2023

Version 2

- (MACHEREY-NAGEL Inc.) V.2
- Version 1 is forked from Total nucleic acid extraction Maxwell(R) HT Environmental TNA Kit, custom (Promega)

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

Total nucleic acid extraction from wastewater using NucleoMag® DNA/RNA Water Kit (Catalog no. 744220.1, MACHEREY-NAGEL Inc., Duren, Germany).

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

- •Ethanol USP/ACS or molecular biology grade (100%)
- •Molecular biology grade water
- •Isopropanol molecular biology grade (100%)
- •6x KingFisher 96-well plates (Cat. no.: 95040460)
- •1x KingFisher 96-tip comb well plate(Cat. no.: 97002534)
- •Screw cap microcentrifuge tubes

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

Equipment	
Kingfisher Flex	NAME
Automated Extraction System	TYPE
ThermoFisher	BRAND
5400630	SKU





Troubleshooting

Before start

- 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. Prepare the 6 purification plates:
- Sample plate: Add 400µL lysate, 25 µL NucleoMag®B-Beads (vortex the bottle at max speed before use), and 475 µL MWA2 into each well required for purification.
 - Wash 1 plate: 850 μL MWA3 to each well required for purification.
 - Wash 2 plate: (same as plate Wash 1): 850 μL MWA3 to each well required for purification.
 - Wash 3 plate: Add 850 μL MWA4 to each well required for purification.
 - **Elution plate**: Add 100 μL RNase-free H2O to each well required for purification.
- Tip plate: Place KingFisher 96-tip comb into an empty KingFisher 96-well plate. While opening the 96-tip comb plate, pay attention to not touch the tips.



Total nucleic acid extraction

2h

1 For **HA filter** extraction, let the sample thaw on ice and go to **step 2**.

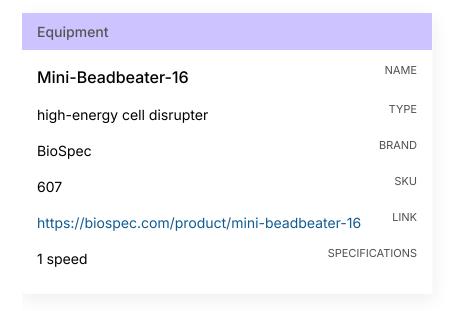
5m

For **BCoV/BRSV** extraction (in duplicate), add 5 μ L of BCoV/BRSV solution to the 2-mL tube containing 475 μ L MWA1. Vortex for 15 seconds (speed 7 out of 10) and flash freeze the tube. Go to step 4.

For **Direct extraction**, add 150 μ L of wastewater to the 2-mL tube containing 100 μ L CTAB. Vortex for 15 seconds (speed 7 out of 10) and flash freeze the tube. Go to step 4.

2

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



2.1 Bead beat for 00:02:30

2m 30s

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 . 7m 30s 3 Centrifuge at maximum speed for 1 min at room temperature. 1m 3 150000 rpm, Room temperature, 00:01:00 4 For **HA filter** extraction, transfer 400 µL of supernatant to the **Lysis/Bind plate**. 10m For BCoV/BRSV/Direct extraction, transfer all supernatant to the Lysis/Bind plate. 5 Start the protocol MN_96_Flex.bdz on the KingFisher Flex 01:14:00 1h 14m Equipment NAME **Kingfisher Flex** TYPE **Automated Extraction System BRAND** ThermoFisher SKU 5400630 6 Transfer the purified sample from the **Elution plate** to the **microcentrifuge tubes**. 10m Note The DNA/RNA is now ready for downstream applications. RNA extract may be stored in RNase-free water at -80°C for 1 year. RT-ddPCR

7 Quantification by Droplet Digital PCR (ddPCR)



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