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Protocol status: Working
We use this protocol and it's working

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🌐 Total nucleic acid extraction - NucleoMag® DNA/RNA Water Kit (MACHEREY-NAGEL Inc.) V.2

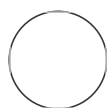
🔗 Version 1 is forked from [Total nucleic acid extraction - Maxwell\(R\) HT Environmental TNA Kit, custom \(Promega\)](#)

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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.

PROTOCOL integer ID:
81243

Keywords: wastewater, SARS-CoV-2, total nucleic extraction, RNA, DNA, extraction, purification, KingFisher

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-beadbeater-16	LINK
1 speed	SPECIFICATIONS

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

 Promega_Maxwell_HT_RNA_Wastewater_V1.bdz

BEFORE START INSTRUCTIONS

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 400 µL 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 µL of BCoV/BRSV solution to the 2-mL tube containing 475 µL MWA1. Vortex for 15 seconds (speed 7 out of 10) and flash freeze the tube. Go to step 4.

For **Direct extraction**, add 150 µL of wastewater to the 2-mL tube containing 100 µL 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.

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

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

 150000 rpm, Room temperature,
00:01:00

4 For **HA filter** extraction, transfer 400 μ L of supernatant to the **Lysis/Bind plate**. 10m
For **B CoV/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

Kingfisher Flex	NAME
Automated Extraction System	TYPE
ThermoFisher	BRAND
5400630	SKU

- 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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