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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VERSION 2

MAY 02, 2023

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q26g7yjq9gwz/v2

Protocol Citation: Adélaïde Roguet, Melissa Schussman 2023. Total nucleic acid extraction - NucleoMag® DNA/RNA Water Kit (MACHEREY-NAGEL Inc.). **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7yjq9gwz/v2Version created by Melissa Schussman

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

Created: May 02, 2023

Last Modified: May 02, 2023

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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 400µL lysate, 25 µL NucleoMag®B-Beads (v*ortex 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.

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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 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

Kingfisher Flex	NAME
Automated Extraction System	TYPE
ThermoFisher	BRAND
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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 -80ºC for 1 year.

RT-ddPCR

7 Quantification by Droplet Digital PCR (ddPCR)

dx.doi.org/10.17504/protocols.io.bpg6mjze