May 02, 2023 Version 2

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)

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

Adélaïde Roguet[1], Melissa Schussman[1]

[1]University of Wisconsin - Milwaukee

mclellan lab

Melissa Schussman
University of Wisconsin - Milwaukee

DOI: https://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/v2**Version created by **Melissa Schussman**

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

Created: May 02, 2023

Last Modified: May 02, 2023

Protocol Integer ID: 81243

Keywords: wastewater, SARS-CoV-2, total nucleic extraction, RNA, DNA, extraction, purification, KingFisher, total nucleic acid extraction from wastewater, rna water kit, total nucleic acid extraction, wastewater, dna, rna, extraction, macherey

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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-beadbeater-16	LINK
1 speed	SPECIFICATIONS

Equipment

Kingfisher Flex	NAME
Automated Extraction System	TYPE
ThermoFisher	BRAND
5400630	SKU

Promega_Maxwell_HT_RNA_Waste...

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 (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.

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.
150000 rpm, Room temperature, 00:01:00

1m

4 For **HA filter** extraction, transfer 400 µL of supernatant to the **Lysis/Bind plate**.
For **BCoV/BRSV/Direct extraction**, transfer all supernatant to the **Lysis/Bind plate**.

10m

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)

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