



APR 05, 2023

OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.q26g7yjq9gwz/v1](https://dx.doi.org/10.17504/protocols.io.q26g7yjq9gwz/v1)

**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/v1)  
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**Protocol status:** Working  
 We use this protocol and it's working

**Created:** Apr 05, 2023

**Last Modified:** Apr 05, 2023

**PROTOCOL integer ID:**  
 80055

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

🔗 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 Maxwell(R) HT Environmental TNA Kit, custom (Promega)

### 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.

**Keywords:** wastewater, SARS-CoV-2, total nucleic extraction, RNA, DNA, extraction, purification, Promega, 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
<a href="https://biospec.com/product/mini-beadbeater-16">https://biospec.com/product/mini-beadbeater-16</a>	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 50% ethanol solution (it must be fresh!)
3. Prepare the 6 purification plates:
  - **Wash 1 plate:** Add 100 µl of 50% ethanol and 900 µl of wash buffer (WBA) to each well required for purification.
  - **Wash 2 plate** (same as plate Wash 1): Add 100 µl of 50% ethanol and 900 µl of wash buffer (WBA) to each well required for purification.
  - **Ethanol Wash plate:** Add 450 µl of 50% ethanol to each well required for purification.
  - **Elution plate:** Add 100 µl of 25 mM Tris-HCl (pH 8.0) 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.*
  - **Lysis and Bind plate:** Add 35 µl of Resin to each well required for purification (*vortex the bottle at max speed before use*). Add 50 µl of Alkaline Protease Solution custom (APA) to each well required for purification. Add 250 µl of cell lysis solution (CLD) to each well required for purification. Add 400 µl of Isopropanol (100%) to each well required for purification.

## 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  $\mu$ L of supernatant to the **Lysis/Bind plate**.  
For **B CoV/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](https://dx.doi.org/10.17504/protocols.io.bpg6mjze)