

# Sep 09, 2021

# ◆ Total nucleic acid extraction - Maxwell(R) HT Environmental TNA Kit, custom (Promega) V.4

# Adélaïde Roguet<sup>1</sup>

<sup>1</sup>UWM





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

mclellan lab



McLellan Lab

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 <a href="protocols.io">protocols.io</a> is not peer reviewed and may not have undergone a formal approval of any kind.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 <a href="protocols.io">protocols.io</a>, 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 Maxwell(R) HT Environmental TNA Kit, custom (Promega)

DOI

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

PROTOCOL CITATION

Adélaïde Roguet 2021. Total nucleic acid extraction - Maxwell(R) HT Environmental TNA Kit, custom (Promega). **protocols.io** 

https://dx.doi.org/10.17504/protocols.io.bujgnujw

Version created by McLellan Lab

## KEYWORDS

null, wastewater, SARS-CoV-2, total nucleic extraction, RNA, DNA, extraction, purification, Promega, KingFisher

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Apr 27, 2021

LAST MODIFIED

Sep 09, 2021

Citation: AdÃÂ@laÃÂ<sup>-</sup>de Roguet (09/09/2021). Total nucleic acid extraction -ÃÂ Maxwell(R) HT Environmental TNA Kit, custom (Promega). <a href="https://dx.doi.org/10.17504/protocols.io.bujgnujw">https://dx.doi.org/10.17504/protocols.io.bujgnujw</a>

PROTOCOL INTEGER ID

49480

PARENT PROTOCOLS

In steps of

Concentration of viruses from sewage using HA filters

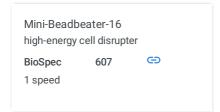
#### **GUIDELINES**

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 TEXT

#### **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



Kingfisher Flex
Automated Extraction System
ThermoFisher 5400630

@ Promega\_Maxwell\_HT\_RNA\_Wastewater\_V1.bdz

# 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 <a href="protocols.io">protocols.io</a> 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 <a href="protocols.io">protocols.io</a>, 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.

## BEFORE STARTING

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  $\mu$ l of 50% ethanol and 900  $\mu$ l of wash buffer (WBA) to each well required for purification.
- **Wash 2 plate** (same as plate Wash 1): Add 100  $\mu$ l of 50% ethanol and 900  $\mu$ l 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  $\mu$ l 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  $\mu$ L of Resin to each well required for purification (vortex the bottle at max speed before use). Add 50  $\mu$ l of Alkaline Protease Solution custom (APA) to each well required for purification. Add 250  $\mu$ l of cell lysis solution (CLD) to each well required for purification. Add 400  $\mu$ l 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**.

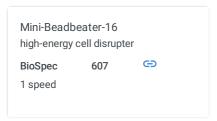
5m

For **BCoV/BRSV** extraction (in duplicate), add  $5 \mu L$  of BCoV/BRSV solution to the 2-mL tube containing 250  $\mu L$  CTAB. Vortex for 15 seconds (speed 7 out of 10) and flash freeze the tube. Go to step 4.

For **Direct extraction**, add 150  $\mu$ L of wastewater to the 2-mL tube containing 100  $\mu$ 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



Start the bead beating when the beads start to be loose in the tubes.

5m

2.2 Cooldown the samples on ice for  $\bigcirc$  **00:05:00** .

protocols.io
3
09/09/2021

3 Centrifuge at maximum speed for 1 min at room temperature. **3150000 rpm, Room temperature**, **00:01:00** 

4 For **HA filter** extraction, transfer 125-250 μL of supernatant to the **Lysis/Bind plate**. For **BCoV/BRSV/Direct extraction**, transfer all supernatant to the **Lysis/Bind plate**.

10m

The default volume transferred is 250 µL. However, for WWTPs with "dirty" influents, we only transfer 125 µL.

5 Start the protocol Promega\_Maxwell\_HT\_RNA\_Wastewater\_V1.bdz on the KingFisher Flex **© 01:14:00** 

1h 14m

Kingfisher Flex

**Automated Extraction System** 

ThermoFisher 5400630

6 Transfer the purified sample from the **Elution plate** to the **microcentrifuge tubes**.

10m

The DNA/RNA is now ready for downstream applications. RNA extract may be stored in RNase-free water at  $-80^{\circ}$ C for 1 year.

## RT-ddPCR

7 Quantification by Droplet Digital PCR (ddPCR)

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