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

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Protocol status: In development

We are still developing and optimizing this protocol

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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.

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...



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 50% ethanol solution (it must be fresh!)
3. Prepare the 6 purification plates:
 - **Wash 1 plate:** Add 100 μ l of 50% ethanol and 900 μ l of wash buffer (WBA) to each well required for purification.
 - **Wash 2 plate** (same as plate Wash 1): Add 100 μ l of 50% ethanol and 900 μ l of wash buffer (WBA) to each well required for purification.
 - **Ethanol Wash plate:** Add 450 μ l of 50% ethanol to each well required for purification.
 - **Elution plate:** Add 100 μ 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 μ L of Resin to each well required for purification (*vortex the bottle at max speed before use*). Add 50 μ l of Alkaline Protease Solution custom (APA) to each well required for purification. Add 250 μ l of cell lysis solution (CLD) to each well required for purification. Add 400 μ 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 μ L of BCoV/BRSV solution to the 2-mL tube containing 250 μ 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 μ L of wastewater to the 2-mL tube containing 100 μ 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.

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  [go to step #2.1](#) .

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


10m

Note

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

1h 14m

 01:14:00

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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