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Protocol for environmental Nucleic Acid (RNA and DNA) extraction from filter sample using MagMAX Microbiome Ultra Nucleic Acid Isolation Kit

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

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Abstract

The objective of this protocol is environmental RNA and DNA (eNA) extraction from water samples filtered through 0.22 µm Sterivex^{TM} filter units or polycarbonate 3.0 µm filter, using the **MagMAX Microbiome Ultra Nucleic Acid Isolation Kit** (Thermo Fisher Scientific)

It was modified by based on a previously established and standardized our protocol for eNA extraction for microbial community analysis.

This protocol is used upstream to molecular biology analysis (e.g. NGS, cDNA library construction, RT-PCR, qPCR) to specifically target microbe eNA.

Guidelines

This protocol has been modified Manual provided by Thermo Fisher Scientific:

- **MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit** (Thermo Fisher Scientific, Catalog Number A42358) (with tube, 100 preps));

MagMAXMicrobiomeNuclAcidIsolat... 1.1MB

Materials

- Samples

- **Sterivex-GP 0.22 µm cartridges** (Merck Millipore, SVGP01050) :
For both RNA and DNA samples, cartridges were filled with 1.6 mL of RNAlater™ Stabilization Solution (Invitrogen™, AM7020) and stored at −20 °C.
For DNA-only samples, cartridges were stored without RNAlater at −80 °C.

- **Nuclpore membranes** (PC MB, 47 mm, 3.0 µm) :
For both RNA and DNA samples, membranes were treated with RNAlater™ Stabilization Solution after that stored at −20 °C.
For DNA-only samples, membranes were stored without RNAlater at −80 °C.

- Reagents

- MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit [with tubes] (Thermo Fisher Scientific, A42358)
- RNase AWAY (Thermo Fisher Scientific, 7002)
- Nuclease-Free Water: Distilled Water, Deionized, Sterile (NIPPON GENE, 316-90101)
- Ethanol(99.5) for Molecular Biology (FUJIFILM, 054-07225)

- Equipments

- Designated RNA work area and Biological Safety Cabinet
- Microsmash Beads Beater (TOMY, MS-100)
- Microcentrifuge for 5 mL and 2mL tubes : Centrifugal force needed: 5000 x g
- Digital VORTEX-GENIE 2 (Scientific Industries, SI-A236)
with 6-inch Platform (Scientific Industries, 146-6005-00) and Microtube Foam Insert (Scientific Industries, 504-0234-00)
- DynaMag-2 (Thermo Fisher Scientific, DB12321)
- Aluminum block thermostatic bath for 2mL tubes : 75°C
- Sterile forceps : For transfering the filter into a 5 mL tube
- Pipettes : 1000µL - 100µL

- Consumables

- Filter tips : 1000µL - 100µL
- 5 mL tubes (TOMY, TM-657S)
- 2 mL tubes (VIOLAMO, 1-1600-04)
- 1.5 mL tubes
- Terumo Syringes 10mL for Vaccination Slip Tip White (TERUMO, SS-10SZ) : For pushing out the RNAlater from the Sterivex cartridge

Before start

- Wear gloves throughout the extraction process.
- Use filter tips to minimize the risk of contaminations.
- All steps have to be conducted within a designated RNA work area.

a) Turn on Biological Safety Cabinet blower. Clean the work surfaces with RNase AWAY (Thermo Fisher Scientific, 7002) to remove nucleases. Then Lower the sash and run the UV light for 30 minutes. Switch the UV light back to white light; you are ready to begin.
b) Precipitates can occur if the Lysis Buffer or Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the reagents at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.
c) Prepare 80% Ethanol from 99.5% absolute Ethanol and Nuclease-Free Water. Prepare enough solution for a minimum volume of 2 mL per sample.

Before each use of the kit
1. Vortex the Beads vigorously to ensure they are homogenous.
2. Prepare Binding/Bead Mix according to the following table:

Component	Volume per well [1]
Total Nucleic Acid Binding Buffer	500 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume	**520 µL**

[1] Use 10% overage calculation when making a master mix for use with multiple samples.

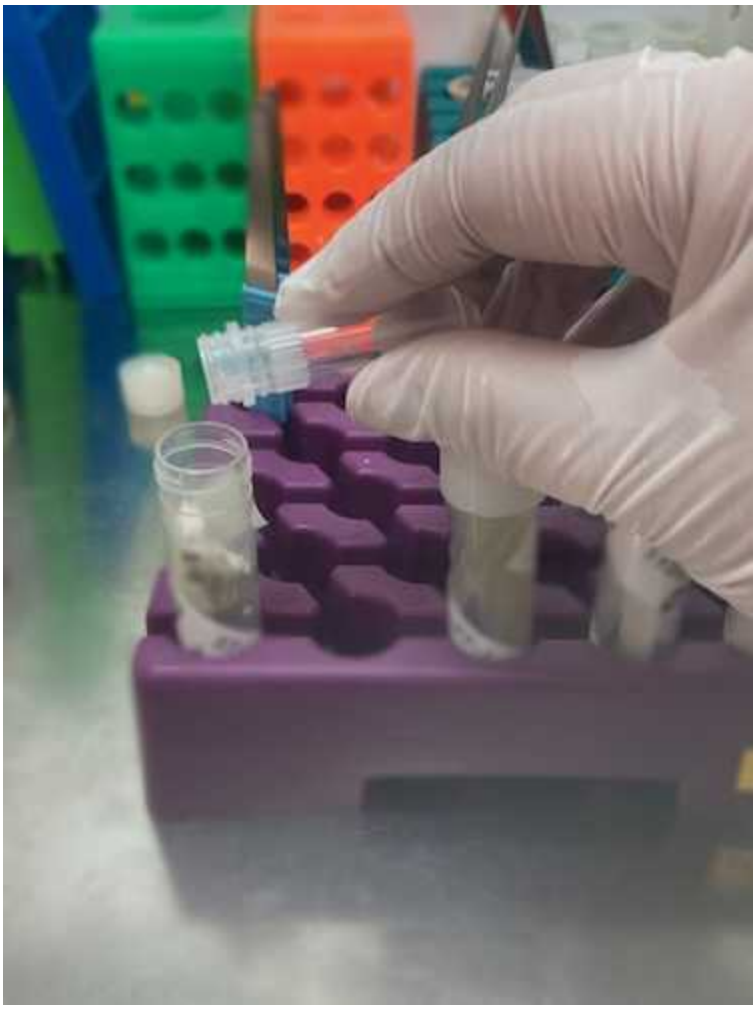
3. Mix well by inversion, then store at room temperature.

Transfer the Sample into the beads tube

1 Push out the RNAlater from the Sterivex cartridge with 10 mL syringe (TERUMO, SS-10SZ).

2 Open the Sterivex cartridg by a tube cutter. Using a surgical scalpel (Akiyama MEDICAL MFG, Replacement blade No. 15) and two sets of sterile forceps, pick up the filter membrane at opposite edges and transfer the filter into a 5 mL tube (TOMY, TM-657S) with the top side inward.

3 Add the **homogenization beads** (provided in the kit) into the 5 mL tube.



Sample lysis

7m 30s

4 Add 800 µL of the **Lysis Buffer** to the 5 mL tubes.

800 µL

5 Place the 5 mL tubes into Microsmash Beads Beater (TOMY, MS-100). Shake at speed 3,800 rpm for 1 minute and then rest for 30 seconds, then 3,800 rpm for 1 minute.

2m 30s

3800 rpm, Room temperature , 00:01:00 00:00:30

3800 rpm, Room temperature , 00:01:00

Equipment

Microsmash Beads Beater	NAME
TOMY	BRAND
MS-100	SKU
https://bio.tomys.co.jp/products/micro_homogenizing_system/	LINK

6 Centrifuge the 5 mL tubes at 5000×g for 5 minutes at room temperature. 5m

5000 rpm, Room temperature, 00:05:00

7 Transfer the supernatant to a clean 2 mL Tube (VIOLAMO MicroTube 1-1600-04). It will be necessary to push the tip of pipett through the beads into the bottom of the tube in order to recover as much supernatant as possible.
Note: However, don't transfer the beads as much as possible.

<STOPPING POINT> Bead tube can be stored at 4°C overnight after lysis.

Bind the Magnetic Beads 10m

8 Invert the **Binding Bead Mix** to mix, then add 520 µL to each tubes.

520 µL

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO

NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.
Note: Centrifuge the tube at 800 rpm for 1 minute to collect liquid to bottom of tube if needed.

9 Shake the tube at 900 rpm for 5 minutes. 5m

900 rpm, Room temperature , 00:05:00

Equipment

Digital VORTEX-GENIE 2	NAME
Scientific Industries, Inc	BRAND
SI-A236	SKU
https://www.scientificindustries.com/digital-vortex-genie-2.html	LINK

Equipment

Tube Support for Vortex GENIE2	NAME
Scientific Industries, Inc.	BRAND
504-0234-00	SKU

Equipment

6 Platform Head, for Vortex GENIE2	NAME
Scientific Industries, Inc.	BRAND
146-6005-00	SKU

10 Place the tube on the magnetic stand for at least 5 minutes, or until all the beads have collected 00:05:00 Room temperature

5m

Equipment

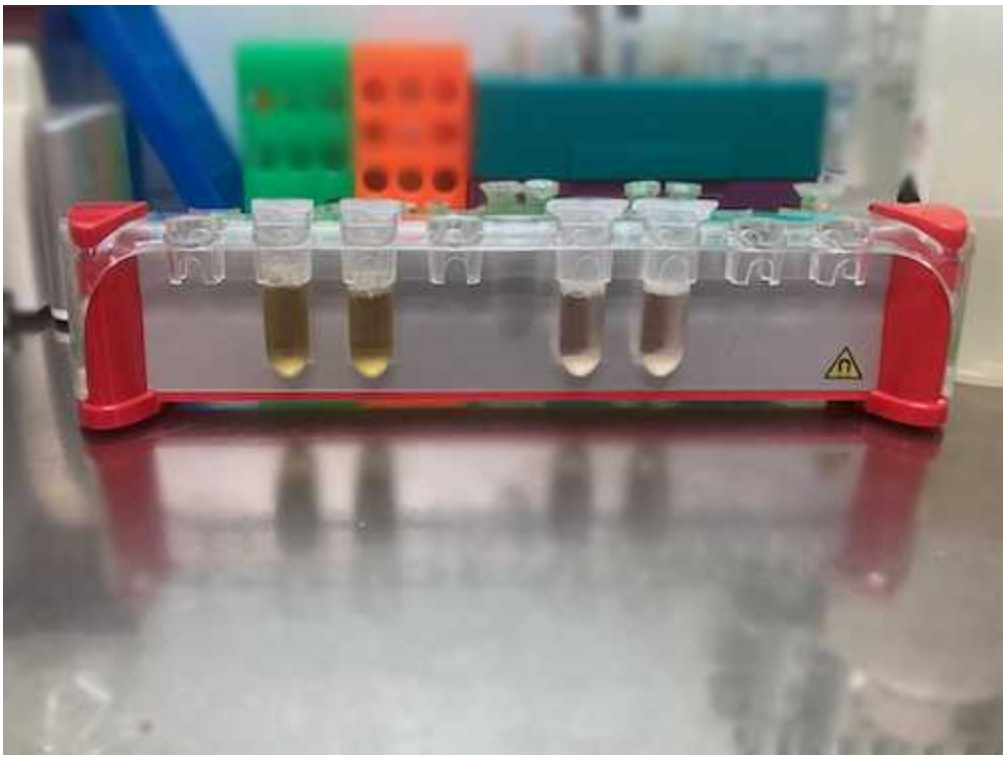
DynaMag™-2 Magnet

Magnetic tube rack

Invitrogen™

12321D

https://www.thermofisher.com/order/catalog/product/12321D#:~:text=The%20DynaMa



Wash the Magnetic Beads

24m

11 Keeping the tube on the magnet, carefully open the lid, then discard the supernatant.
Note: Be sure to completely remove any contaminating homogenization beads.

IMPORTANT! Avoid disturbing the magnet beads.

12 Remove the tube from the magnetic stand, then add 1 mL of the **Wash Buffer** to each sample.

1 mL

13 Shake at 800 rpm for 30 seconds.

30s

800 rpm, Room temperature , 00:00:30

Equipment

Digital VORTEX-GENIE 2	NAME
Scientific Industries, Inc	BRAND
SI-A236	SKU
https://www.scientificindustries.com/digital-vortex-genie-2.html	LINK

Equipment

Tube Support for Vortex GENIE2	NAME
Scientific Industries, Inc.	BRAND
504-0234-00	SKU

Equipment

NAME	BRAND	SKU
6 Platform Head, for Vortex GENIE2	Scientific Industries, Inc.	146-6005-00

14 Place the tube on the magnetic stand for 3 minutes, or until all the beads have collected. 3m
00:03:00

Equipment

DynaMag™-2 Magnet

Magnetic tube rack

Invitrogen™

12321D

https://www.thermofisher.com/order/catalog/product/12321D#:~:text=The%20DynaMa

15 Keeping the tube on the magnet, carefully open the lid, then discard the supernatant from each well.
IMPORTANT! Avoid disturbing the magnet beads.

16 Repeat from step 12 to step 15 using 1 mL of the **Wash Buffer**. 3m 30s
1 mL 00:03:30

17 Repeat from step 12 to step 15 using 1 mL of **80% Ethanol**. 3m 30s
1 mL 00:03:30

18 Repeat from step 12 to step 15 using 1 mL of **80% Ethanol**. 3m 30s

1 mL 00:03:30

19 Dry the beads with open the cap for 10 minutes at room temperature. 10m

00:10:00 Room temperature

Elute the nucleic acid 13m

20 Add 50 µL of the **Elution Solution** to each tubes.

50 µL

21 Place the tube on heat-block at 75°C for 5 minutes. 5m

75 °C 00:05:00

22 Place the tube on a shaker at 800 rpm for 5 minutes. 5m

800 rpm, Room temperature , 00:05:00

Equipment

Digital VORTEX-GENIE 2	NAME
Scientific Industries, Inc	BRAND
SI-A236	SKU
https://www.scientificindustries.com/digital-vortex-genie-2.html	LINK

Equipment

Tube Support for Vortex GENIE2	NAME
Scientific Industries, Inc.	BRAND
504-0234-00	SKU

Equipment

6 Platform Head, for Vortex GENIE2	NAME
Scientific Industries, Inc.	BRAND
146-6005-00	SKU

23 Place the tube on the magnetic stand for 3 minutes or until all beads are collected against the magnets. 3m

00:03:00

Equipment

DynaMag™-2 Magnet

Magnetic tube rack

Invitrogen™

12321D

https://www.thermofisher.com/order/catalog/product/12321D#:~:text=The%20DynaMa

24 Keep the tube on the magnet and carefully open the lid, then transfer the eluates to a new 1.5 mL tube.

25 The purified nucleic acid is ready for immediate use. Alternatively, store at −20°C for up to 6 months and −80°C for greater than 6 months.

Options

26 For DNA sample: Phenol-chloroform extraction (PCI purification) is performed after RNase treatment.
For RNA sample: TURBO DNA-*free*™ Kit (Invitrogen, AM1907) is performed.