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Protocol for environmental Nucleic Acid (RNA and DNA) extraction from filter sample using MagAttract PowerWater DNA/RNA Kit

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We use this protocol and it's working

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Abstract

This protocol describes the extraction of environmental RNA and DNA (eNA) from water samples filtered through 0.22 µm Sterivex filter units or 3.0 µm polycarbonate filters using the MagAttract PowerWater DNA/RNA Kit (QIAGEN, 27800-4-EP).
The procedure has been modified for manual handling and employs a Microsmash Beads Beater (MS-100).
The extracted eNA can be used for downstream molecular analyses such as next-generation sequencing (NGS), cDNA library construction, RT-PCR, and qPCR to specifically target microbial eNA.

Guidelines

This protocol has been modified Manual provided by QIAGEN:

-MagAttract®PowerWater® DNA/RNA Kit(QIAGEN)

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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 RNAlate™ Stabilization Solution after that stored at −20 °C.
For DNA-only samples, membranes were stored without RNAlater at −80 °C.

- Reagents

- MagAttract®PowerWater® DNA/RNA Kit(384) (QIAGEN, 27800-4-EP)
- RNase AWAY (Thermo Fisher Scientific, 7002)
- 2-Mercaptoethanol (β-ME:beta-mercaptoethanol) (SIGMA, M3148-25ML)
- 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 : Centrifugal force needed: 4500 x g
- Digital VORTEX-GENIE 2 (Scientific Industries, SI-A236)
- DynaMag™-5 Magnet (Thermo Fisher Scientific, 12303D)
- Aluminum block thermostatic bath for 5mL tubes : 60 - 65°C
- Sterile forceps : For transfering the filter into a 5 mL tube
- Pipettes : 1000µL - 100µL

- Consumables

- Filter tips : 1000µL - 100µL
- 5 mL tubes (WATSON 233-150C)
- 1.5 mL tubes
- Terumo Syringes 10mL for Vaccination Slip Tip White (TERUMO, SS-10SZ) : For pushing out the RNAlater from the Sterivex cartridge
- Parafilm

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.

Before Starting

15m

1 Warm **Solution MBL** at 60°C for 15-20 minutes to dissolve precipitates. 15m

60 °C 00:15:00

2 Warm Clear-Mag **RNase-Free Water** at 65°C.

65 °C

3 To extract both DNA and RNA or only RNA, add 25µL of β-mercaptoethanol (β-ME) to 975 µL of Solution MBL.

25 µL

Sample Transfer to Bead Tube

4 Expel the RNAlater from the Sterivex cartridge using a 10 mL syringe (TERUMO, SS-10SZ).

5 Cut open the Sterivex cartridge with a tube cutter. Using a surgical scalpel (Akiyama MEDICAL MFG, Replacement blade No. 15) and two sets of sterile forceps, grasp the filter membrane at opposite edges and transfer the filter into a **5 mL PowerWater DNA Bead Tube** (provided) with the top side (sample side) facing inward.

Sample Lysis

20m 35s

6 Add 1.5 mL of pre-warmed **Solution MBL** to each PowerWater DNA Bead Tube.

1.5 mL

7 Place the **PowerWater DNA Bead Tubes** into a Microsmash Beads Beater (TOMY, MS-100). Disrupt the sample at 3,800 rpm for 1 minute, rest for 30 seconds, and then repeat disruption again at 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

8 Centrifuge the **PowerWater DNA Bead Tubes** at 4500 x g for 1 minute at room temperature. 4500 x g, Room temperature, 00:01:00 1m

9 Transfer the supernatant to a clean 5 mL tube (WATSON 233-150C). To recover as much supernantant as possible, insert the pipette tip through the beads to the bottom of the bead tube.
Note: The supernatant may still contain some bio-solid particles.

10 Add 200 µL of **Solution IRS** to each tube and seal with Parafilm.
200 µL

11 Vortex horizontally at 500 rpm for 5 seconds to ensure that the solution is well mixed.
500 rpm, Room temperature , 00:00:05 5s

Equipment

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

12 Incubate at room temperature for 5 minutes. 5m
Room temperature 00:05:00

13 Centrifuge the 5 mL tubes at 4500 x g for 6 minutes at room temperature . 6m
4500 x g, Room temperature, 00:06:00

14 Avoiding the pellet, transfer the entire volume of supernatant to a new 5 mL tube.

15 Centrifuge again at 4500x g for 6 minutes to remove any residual particulates that may have carried over. 6m
4500 x g, Room temperature, 00:06:00

16 Transfer all supernatant (~1 mL) to a new 5 mL tube (WATSON 233-150C), carefully avoiding any residual pellet.

<STOPPING POINT> If needed, store the supernatant in the 5ml tube at 4°C for several hours before continuing the protocol.

Binding the Magnetic Beads 23m

17 Ensure **ClearMag Beads** are thoroughly resuspended by shaking or rocking the bottle, and re-agitate the beads every 3 minutes.

18 For each preparation, add 20 µL of **ClearMag Beads** and 1 mL of **ClearMag Binding Solution**. Seal the tube with Parafilm.
1 mL 20 µL

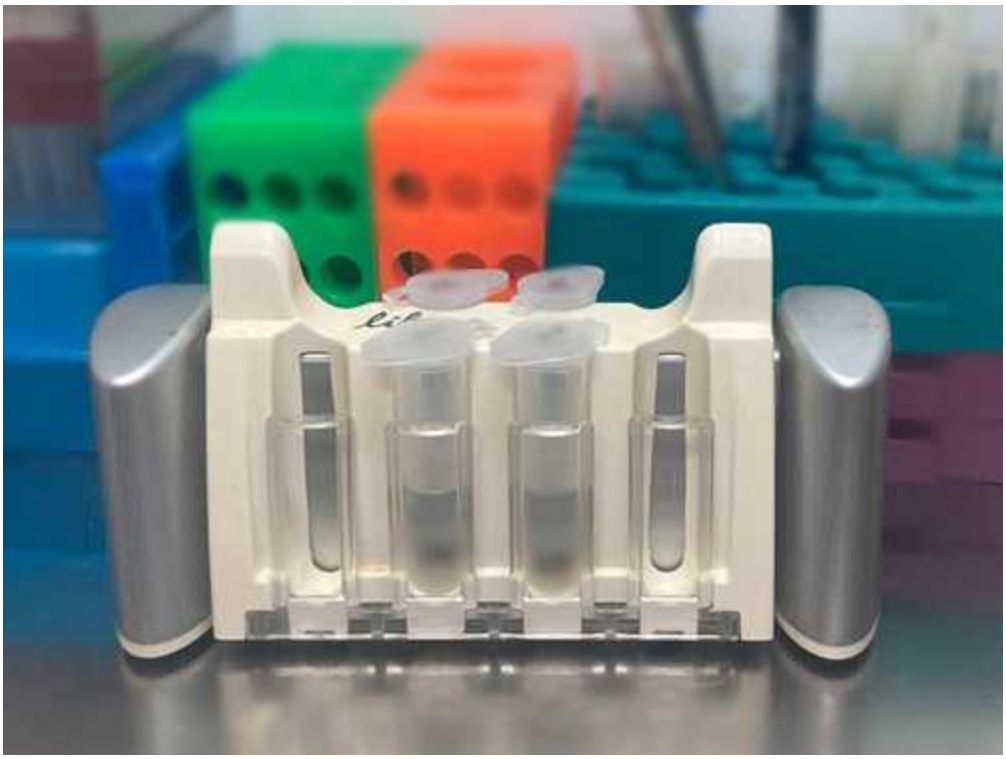
19 Mix the beads and the lysate by placing the tube horizontally on a vortex at the lowest setting (VORTEX-GENIE 2 = 500 rpm) for 20 minutes to allow DNA binding. 20m
500 rpm, Room temperature , 00:20:00

Equipment

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

20 Place the tube on a magnetic stand and allow about 3 minutes for the beads to completely adhere to the magnet. 3m

00:03:00



Equipment

DynaMag™-5 Magnet	NAME
Invitrogen™	BRAND
12303D	SKU
https://www.thermofisher.com/order/catalog/product/12303D?SID=srch-srp-12303D	LINK

21 Carefully remove and discard the supernatant without disturbing the beads.

Washing the Magnetic Beads

29m

22 Add 500 µL of **ClearMag Wash Solution** and remove the tube from the magnetic stand.

500 µL

23 Resuspend the beads thoroughly by pipetting up and down for 10 times or by vortexing at the lowest speed for 10 sec, until all beads are fully resuspended.

24 Place the tube on the magnetic stand and allow 3 minutes for the beads to adhere to the magnet. Carefully remove the supernantant.

3m

00:03:00

Equipment	
DynaMag™-5 Magnet	NAME
Invitrogen™	BRAND
12303D	SKU
https://www.thermofisher.com/order/catalog/product/12303D?SID=srch-srp-12303D	LINK

25 Repeat steps 21 - 23 two more times (for a total of 3 washes) using 500 µl of ClearMag Wash Solution each time. 6m

500 µL 00:03:00

26 Using a 200 µL pipette tip, carefully remove any remaining wash from the tube, taking care not to disturb the beads.

27 To ensure all wash solution is removed, leave the tubes open in a biosafety hood for 5 minutes, place them on the magnetic stand to remove any remaining liquid, and dry again for 5 mins or until the beads are visibly dry. 20m

00:20:00

Elution of Nucleic Acid 8m

28 Resuspend the beads in 100 µL (both RNA and DNA) or 50 µl (RNA or DNA only) of **Clear-Mag RNAse-free water** to elute the nucleic acid.

100 µL

29 To elute the nucleic acid, heat at 65°C for 5 minutes, flicking the tube occasionally to mix. 5m

65 °C 00:05:00

30 Place the tube on the magnetic stand and allow 3 minutes for the beads to adhere to the magnet. 3m

00:03:00

Equipment

DynaMag™-5 Magnet	NAME
Invitrogen™	BRAND
12303D	SKU
https://www.thermofisher.com/order/catalog/product/12303D?SID=srch-srp-12303D	LINK

31 Keep the tube on the magnetic stand and carefully open the lid, then transfer the eluate to a new 1.5 mL tube.

32 The purified nucleic acids can be used immediately. Alternatively, store the DNA and RNA at -80°C for long-term preservation.

Options

33 For DNA samples: Proceed to the RNase digestion step. To remove RNA contamination, add 2 µL of RNase A (10 mg/mL, Fermentas) to 20 µL of DNA and incubate for 3–4 hours at 37 °C. After enzyme digestion, remove the RNase A using Macherey-Nagel's NucleoSpin gDNA Clean-up Kit, following the manufacturer's protocol.
For RNA samples, proceed to the DNAse digestion step using TURBO DNA-*free*™ Kit (Invitrogen, AM1907) and cDNA library preparation steps using PrimeScript™ II 1st strand cDNA Synthesis Kit (TaKaRa, 6210A) .