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Ceres Nanoparticles Concentration and Extraction using MagMAX Wastewater kit

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Shannon Fitz[1], Alex Shaw[1], Dilip Abraham[2], michael Owusu[3]

[1]Imperial College London; [2]CMC Vellore; [3]Kwame Nkrumah University of Science and Technology

GEMS - Genomic Environ…

Shannon Fitz
Imperial College London

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

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Abstract

Ceres Nanotrap Microbiome A Particles are used to capture and concentrate important pathogens from samples. https://www.ceresnano.com/protocols Protocol APP-082 was used to concentrate and extract target pathogens, scaled up from 10 mL to 35 mL samples using Ceres Microbiome A particles and the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. Following discussions with Ceres scientists, Enhancement Reagent 3 is recommended as opposed to Enhancement Reagent 1.

Materials

- Nanotrap Magnetic Virus Particles (10) **Ceres Nano Catalog #**44202
- Nanotrap® Enhancement Reagent 3 (ER3) **Ceres Nano Catalog #**10113-10
- Nanotrap® Buffer 2 **Ceres Nano Catalog #**10102-100
- MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit **Thermo Fisher Scientific Catalog #**A52606

Total cost per sample: £19.63
Wastewater kit (100 preps): £3.13 per sample
Enhancement Reagent 3 (10 ml): £1.97 per sample.
Ceres A beads (10 ml): £13.68 per sample.
Nanotrap Buffer (100 ml): £0.85 per sample.

Extra equipment required:
Heat block, horizontal shaker or 50 mL tube rotator, 50 mL tube magnetic rack, 1.5-2 mL tube magnetic rack, vortex, serological pipet, mini centrifuge.

Approximate time needed:
About 2 hours for concentration and extraction (may vary depending on number of samples).

Concentration and extraction

1 **Manual Ceres Nanotrap concentration using Nanotrap Microbiome A Particles for a 35 mL environmental sample**

1.1 Invert the environmental water sample 5 times to mix. Then, let it sit for 45 seconds at room temperature. (No need to wait for samples to reach room temperature before processing).

1.2 Agitate, then filter environmental water sample through a coffee filter to remove suspended solids. Transfer 35 mL of filtered sample into a clean 50 mL conical tube.

1.3 Add 100 µL of Nanotrap Enhancement Reagent 3 (ER3) to the sample, cap the tube, and then invert 2 times to mix.

1.4 Add 525 µL of Nanotrap Microbiome A Particles (Nanotrap particles) to the sample, cap the sample and then invert 2 times to mix the particles.

1.5 Incubate samples with Nanotrap particles at room temperature for 30 minutes.

Note: Invert every 5 minutes or use a rotator.

1.6 Place the tube on a DynaMag-50 magnetic rack to separate the Nanotrap particles from the sample for 10 minutes.

1.7 Using a serological pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

Note: Can use a P-1000 or P-200 pipette to remove any remaining supernatant from the sample (be careful to not lose any Nanotrap particles when removing supernatant).

1.8 Add 1 mL of Nanotrap Buffer 2 to the tube and re-suspend the Nanotrap particle pellet by pipetting on the walls of the conical tube, gently re-suspend until all Nanotrap particles have been completely collected.

1.9 Transfer the Nanotrap particle suspension to a new 2 mL microcentrifuge tube.

1.10 Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.

1.11 Using a P-1000 pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

Note: If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.

1.12 Add 500 µL of MagMAX™ Microbiome Lysis Solution to Nanotrap particle pellet, pipette up and down until Nanotrap particles are resuspended completely.

1.13 Close the tube lid and incubate the samples on a heating block at 56°C for 10 minutes.

1.14 Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.

Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.

1.15 Transfer 400 µL of supernatant/lysate to a new 2 mL collection tube and discard the Nanotrap particles pellet.

1.16 Sample is now ready for Part 2.

1.17 **Part 2: Nanotrap Microbiome A MagMAX Wastewater Extraction Procedure**

1.18 Add 530 µL of MagMAX Binding Buffer to the sample/lysate.

1.19 Add 10 µL of MagMAX Proteinase K to the sample/lysate.

1.20 Add 20 µL of MagMAX Magnetic Beads to the sample/lysate.

1.21 Vortex to mix, then incubate at 65^{0}C on a heat block for 10 minutes.

1.22 Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant using a pipette.

Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.

1.23 Add 1000 µL of MagMAX wash buffer to sample and re-suspend the magnetic beads using a pipette.

1.24 Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant.

1.25 Add 1000 µL of 80% EtOH to sample and re-suspend the magnetic beads using a pipette.

1.26 Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant.

1.27 Add 500 µL of 80% EtOH to sample and re-suspend the magnetic beads using a pipette.

1.28 Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant by using a pipette.

1.29 Centrifuge the tube (Mini Centrifuge at 2000 g for 30 seconds).

1.30 Place the tube on a DynaMag-2 magnetic rack, then remove excess 80% EtOH using a smaller pipette.

1.31 Add 100 µL of MagMAX Elution Solution to re-suspend the magnetic beads and then incubate at 65^{o}C for 10 minutes on a heat block (close caps).

1.32 Place the tube in the DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes.

Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops/condensation from inside the lid before magnetic separation.

1.33 Transfer the supernatant to a new tube, the sample is ready for downstream analysis or can be stored at -80^{o}C.

Note: Multiple freeze-thaw cycles may cause degradation.

Protocol references

Based on the protocol APP-082 from Ceres Nanosciences.