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Version 2

Ceres Nanoparticles Concentration and Extraction using MagMAX Wastewater kit V.2

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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.

This protocol was updated from a previous version to correct an error in costing.



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: £42.08

Wastewater kit (100 preps): £3.13 per sample

Enhancement Reagent 3 (10 ml): £3.00 per sample.

Ceres A beads (10 ml): £35.08 per sample.

Nanotrap Buffer (100 ml): £0.87 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 μ L of Nanotrap Enhancement Reagent 3 (ER3) to the sample, cap the tube, and then invert 2 times to mix.
- 1.4 Add 525 μ L 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°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 μ L 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 μ L 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 μ L 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 μ L of MagMAX Elution Solution to re-suspend the magnetic beads and then incubate at 65°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°C.

Note: Multiple freeze-thaw cycles may cause degradation.

Protocol references

Based on the protocol APP-082 from Ceres Nanosciences.