Nanotrap® Microbiome B; 35 mL Automated Protocol with NucleoMag® Kit and the KingFisher™ Apex

Objective: This protocol uses Nanotrap Microbiome B Particles and Nanotrap Enhancement Reagent 3 to capture and concentrate microbes in environmental water samples. It is optimized for microbe capture from 35 mL samples and is compatible with MACHEREY-NAGEL NucleoMag® DNA/RNA Water Kit. The automated script can process up to 24 samples at once and can be amended for the throughput in your lab.

Materials and equipment:

Sample Type	
Environmental Water Samples	
Concentration Reagent	**Vendor**
Nanotrap Microbiome B Particles	Ceres Nanosciences; SKU# 65202
Nanotrap Enhancement Reagent 3 (ER3)[1]	Ceres Nanosciences; SKU# 10113
Extraction Kit	**Vendor**
NucleoMag DNA/RNA Water Extraction Kit	MACHEREY-NAGEL; REF 744220.1
Materials/Equipment	**Vendor**
KingFisher™ Apex with 96 DW Head	Thermo Fisher Scientific; Cat# 5400930
KingFisher Apex 24 Combi head	Thermo Fisher Scientific; Cat# 24079940
KF Apex 96 KF heating block	Thermo Fisher Scientific; Cat# 24075920
KF Apex 24 DW heating block	Thermo Fisher Scientific; Cat# 24075940
KingFisher 24 Deep-well Plate, Barcoded	Thermo Fisher Scientific; Cat#95040470B
KingFisher 24 Deep-Well Tip Comb & Plate, Barcoded	Thermo Fisher Scientific; Cat#97002610B
KingFisher 96 Deep-well Plate, Barcoded	Thermo Fisher Scientific; Cat# 95040450B
KingFisher 96 Plate (200 µL), Barcoded	Thermo Fisher Scientific; Cat# 97002540B
KingFisher 96 Deep-well Tip Comb, Barcoded	Thermo Fisher Scientific; Cat# 97002534B
General Reagents	**Vendor**
Molecular grade water	VWR; 45001-044

[1] Precipitate can form in ER3 if stored below room temperature. Allow ER3 to return to room temperature to dissolve the precipitate (can invert 2-3 times to help resuspend, do not heat).

Capture and Extract Microbes using Nanotrap Microbiome Particles

Procedure:

1. **Nanotrap Microbiome B NucleoMag KingFisher Apex 35mL Procedure-Part 1**

    1. *Prepare NT Sample B*
        1. Pipette 34.375 mL of environmental water sample into a clean 50 mL conical tube.
        2. To each sample add 100 µL of Nanotrap Enhancement Reagent 3 (ER3) and then invert 2 times to mix it.
        3. Add 525 µL of Nanotrap® Microbiome B Particles to the sample and then invert 2 times to mix the particles.
    2. *Prepare* "Sample Plates 1-7"
        1. Use a repeater pipettor/serological pipette to add 5 mL of NT sample B (35 mL total) to 7 KingFisher 24 Well Deep Well Plates.
            a) We recommend the Eppendorf Repeater E3 or ali-Q™ 2 Aliquoting Pipet Controller to pipette the 5 mL aliquots using 50 mL tips, this reduces handling time and tip usage.
                a. For example: Aspirate the entire 35 mL sample use the 5 mL aliquot function to dispense into the KingFisher plates.
            b) The location for one sample should be the same across all 7 plates.
                a. For example: if sample 1 was added to well A1 for plate 1, sample 1 should also be added to well A1 for plate 2, and so on for all 7 plates.
            c) Add a **Tip Comb** into Sample Plate 1.

    3. *Prepare* "Lysis Plate"
        1. Add 500 µL of Lysis Buffer MWA1 to a new (the 8th) KingFisher 24 Well Deep Well Plate matching the number and location of the "Sample Plate" wells.

    4. *Run NT Script (Request file at sales@ceresnano.com)*
        1. Run **NT_Microbiome_B_NucleoMag_24_w_heat_35mL.kfx**
        2. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

    5. Once the protocol is completed, the "Lysis Plate" will contain lysate that is ready to proceed to Part 2 (***caution* sample may be hot***).

2. **Nanotrap Microbiome B NucleoMag KingFisher Apex**
    1. Prepare “NM Binding” Plate
        1. To a new KingFisher 96 Deep Well Plate, add 450 µL of the cleared lysate (NT lysate) from each well of the lysis plate used in “Part 1 step 5” of the protocol. Keep track of which well contains which sample in this new bead binding plate.
        2. Add 475 µL of Binding Buffer MWA2 to each well in which lysate was added.
        3. Vortex the NucleoMag B-beads thoroughly and add 25 µL to each well.
            a) Note: Binding mix (MWA2 + B-beads) can be pre-mixed before their addition to the plate.
    2. Prepare “1st Wash MWA3” Plate
        1. Add 850 µL of Wash Buffer MWA3 to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher 96 Deep Well Plate- “NM Binding” Plate wells.
    3. Prepare “2nd Wash MWA3” Plate
        1. Add 850 µL of Wash Buffer MWA3 to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher 96 Deep Well Plate- “NM Binding” Plate wells.
    4. Prepare “3rd Wash MWA4” Plate
        1. Add 850 µL of Wash Buffer MWA4 to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher 96 Deep Well Plate- “NM Binding” Plate wells.
    5. Prepare “Elution” Plate
        1. Add 100 µL of Rnase-free water to a new KingFisher 96- 200 µL plate matching the number and location of the KingFisher 96 Deep Well Plate- “NM Binding” Plate wells.
    6. Prepare “Tip Plate”
        1. Insert the KingFisher™ 96 Deep Well Comb into a new KingFisher 96 Deep Well Plate
    7. *Run Extraction Script (Request file at sales@ceresnano.com)*
        1. Run **NucleoMag_DNA_RNA_Water_CeresNanoTrap_Apex_Rev02.kfx**
        2. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

3. Once the protocol is completed, the KingFisher 96-Elution Plate contains eluates that are ready for downstream analysis or can be stored at -80ºC.
    Note: Multiple freeze-thaw cycles may cause degradation.

Attachments: 2

KingFisher™ Apex

1. *NT_Microbiome_B_NucleoMag_24_w_heat_35mL.kfx*
2. *NucleoMag_DNA_RNA_Water_CeresNanoTrap_Apex_Rev02.kfx*