Nanotrap® Microbiome Combined; 10 mL Automated Protocol using NucleoMag® Kit and the KingFisher™ Apex

Objective: This protocol uses Nanotrap Microbiome A Particles and 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 10 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 A Particles	Ceres Nanosciences; SKU# 44202
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 Combined NucleoMag KingFisher Apex Procedure-Part 1**

    1. *Prepare* "Sample Plate 1" *and* "Sample Plate 2"
        1. Invert environmental water sample 5 times to mix. After inverting, place on a flat surface for 45 seconds.
        2. Add 4,800 µL of environmental water sample to one well (one well per sample) of a new KingFisher 24 Well Deep Well Plate.
        3. Add another 4,800 µL of environmental water sample to the same well location on a second KingFisher™ 24 Well Deep Well Plate.
            a) For example, if you loaded a sample into well A1 of the first plate, load the second volume of that sample into well A1 of the second plate.
        4. Add 50 µL of Nanotrap Enhancement Reagent 3 (ER3) Solution to each sample on the two KingFisher 24 Well Deep Well sample plates (100 µL total).
        5. Add 75 µL of Nanotrap® Microbiome A Particles to each sample on the two KingFisher 24 Well Deep Well sample plates (150 µL total).
        6. Add 75 µL of Nanotrap Microbiome B Particles to each sample on the two KingFisher 24 Well Deep Well sample plates (150 µL total).

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

    3. *Prepare* "Tip Plate"
        1. Insert a new tip comb into a new KingFisher 24 Well Deep Well Plate.

    4. *Run NT Script (Request file at sales@ceresnano.com)*
        1. Run **NT_Microbiome_A_and_B_NucleoMag®_24_w_heat.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 Combined NucleoMag KingFisher Apex Procedure-Part 2**

    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 KingFishe 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 Kit 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_A_and_B_NucleoMag®_24_w_heat.kfx*
2. *NucleoMag_DNA_RNA_Water_CeresNanoTrap_Apex_Rev02.kfx*