

Nanotrap[®] Microbiome Combined; 35 mL Automated Protocol with 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 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 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 35 mL Procedure-Part 1

1. Prepare NT Sample AB

1. Pipette 33.850 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 A Particles and 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 AB (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 MWA1 Lysis Buffer 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_A_B_NucleoMag_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 A NucleoMag KingFisher Apex

1. Prepare “NM Binding” Plate
 1. To a new KingFisher 96 Deep Well Plate, add 450 μ L 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 μ L of Binding Buffer MWA2 to each well in which lysate was added.
 3. Vortex the NucleoMag B-beads thoroughly and add 25 μ L 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 μ L 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 μ L 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 μ L 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 μ L of Rnase-free water to a new KingFisher 96- 200 μ L 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 -80°C.

Note: Multiple freeze-thaw cycles may cause degradation.

Attachments: 2

KingFisher™ Apex

1. *NT_Microbiome_A_B_NucleoMag_w_heat_35mL.kfx*
2. *NucleoMag_DNA_RNA_Water_CeresNanoTrap_Apex_Rev02.kfx*