Nanotrap® Microbiome A; 10 mL Automated Protocol with KingFisher™ Apex and ZymoBIOMICS® MagBead DNA/RNA Kit

Objective: This protocol uses Nanotrap Microbiome A Particles and Nanotrap Enhancement Reagent 2 to capture and concentrate microbes in environmental water samples. It is optimized for microbe capture from 10 mL samples and is compatible with ZymoBIOMICS® MagBead DNA/RNA Kit.

Materials and equipment:

Sample Type	
Environmental Water Samples	
Concentration Reagent	**Vendor**
Nanotrap Microbiome A Particles	Ceres Nanosciences; SKU# 44202
Nanotrap Enhancement Reagent 2 (ER2)[1]	Ceres Nanosciences; SKU# 10112
Extraction Kit	**Vendor**
ZymoBIOMICS® MagBead DNA/RNA Kit	Zymo; Cat# R2135
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**
Ethanol	VWR; 1006-012
Molecular grade water	VWR; 45001-044

[1] Precipitate can form in ER2 if stored below room temperature. Allow ER2 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 A ZymoBIOMICS® MagBead Apex Procedure-Part 1**

    1. *Prepare* “Sample Plates 1” *and* “Sample Plates 2”
        1. Invert environmental water sample 5 times to mix. After inverting, place on a flat surface for 45 seconds.
        2. Add 4,875 µL of environmental water sample to one well (one well per sample) of a new KingFisher 24 Well Deep Well Plate.
        3. Add another 4,875 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 2 (ER2) 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).

    2. *Prepare* “Lysis Plate”
        1. Add 400 µL of Zymo Lysis Buffer 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_ZymoBIOMICS_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 A ZymoBIOMICS® MagBead Apex Procedure-Part 2**

    1. *Prepare Zymo Bead Binding Plate*
        1. To a new KingFisher™ 96 Deep Well Plate, add 400 µL of the lysate from each well of the lysis plate used in Part 1 of the protocol. Keep track of which well contains which sample in this new bead binding plate.
        2. Add 400 µL of 100% Ethanol to each well in which lysate was added.
        3. Vortex the Zymo MagBinding Beads thoroughly to homogenize and add 30 µL to each well.

4. Insert the KingFisher™ 96 Deep Well Comb into the Bead Binding Plate.

2. *Prepare Wash Plate 1*
    1. Add 500 µL of Wash buffer 1 to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- Bead Binding Plate wells.
3. *Prepare Wash Plate 2*
    1. Add 500 µL of Wash buffer 2 to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- Bead Binding Plate wells.
4. *Prepare Wash Plate 3*
    1. Add 500 µL of 100% Ethanol to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- Bead Binding Plate wells.
5. *Prepare Wash Plate 4*
    1. Add 500 µL of 100% Ethanol to a new KingFisher™ 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- Bead Binding Plate wells.
6. *Prepare Elution Plate*
    1. Add 100 µL of Zymo DNase/RNase-free water to a new KingFisher™ 96 Well (200 µl) Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- Bead Binding Plate wells.
7. *Run NT Script (Request file at sales@ceresnano.com)*
    1. Run **Zymo_96**
    2. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.
8. Once the protocol is completed, the KingFisher™ 96 Well Elution Plate will contain purified viral RNA that is ready to be loaded onto a PCR plate

Attachments: 2

KingFisher™ Apex

1. *NT_Microbiome_A_ ZymoBIOMICS _24_w_heat.kfx*
2. *Zymo _96*