Nanotrap® Microbiome A; 10 mL Automated Protocol with MagMAX™ Kit and the KingFisher™ Flex

Objective: This protocol uses Nanotrap Microbiome A Particles and Nanotrap Enhancement Reagent 1 to capture and concentrate microbes in environmental water samples. It is optimized for microbe capture from 10 mL samples and is compatible with MagMAX Microbiome Ultra Nucleic Acid Isolation 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 Enhancement Reagent 1 (ER1)[1]	Ceres Nanosciences; SKU# 10111
Extraction Kit	**Vendor**
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit	Thermo Fisher Scientific™; Cat# A42357
Materials/Equipment	**Vendor**
KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head	Thermo Fisher Scientific™; Cat# 5400630
KingFisher™ Flex 24 Deep Well head	Thermo Fisher Scientific™; Cat# 24074440
KingFisher™ Flex 24 Deep Well heating block	Thermo Fisher Scientific™; Cat# 24075440
KingFisher™ Flex 96 heating block	Thermo Fisher Scientific™; Cat# 24075420
KingFisher™ 24 deep-well plate (for Duo Prime, Flex and Presto)	Thermo Fisher Scientific™; Cat# 95040470
KingFisher™ 24 deep-well tip comb and plate (for Flex and Presto)	Thermo Fisher Scientific™; Cat# 97002610
KingFisher™ 96 deep-well plate, v-bottom, polypropylene (for Duo Prime, Flex and Presto)	Thermo Fisher Scientific™; Cat# 95040450
KingFisher™ 96 tip comb for deep-well magnets, 10 x 10 pcs/box (for Flex and Presto)	Thermo Fisher Scientific™; Cat# 97002534
General Reagents	**Vendor**
Ethanol	VWR; 1006-012
Molecular grade water	VWR; 45001-044

[1] Precipitate can form in ER1 if stored below room temperature. Allow ER1 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 MagMAX KingFisher Flex 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 1 (ER1) Solution to each sample on the two KingFisher 24 Well Deep Well sample plates (100 µL total).
        5. Add 75 µL of NanotrapMicrobiome A 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 MagMAX Microbiome Lysis Solution 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_MagMAX_24_w_heat_Flex_10mL.bdz**
        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 MagMAX KingFisher Flex**

    1. *Prepare MagMAX Bead Binding Plate*
        1. To a new KingFisher 96 Deep Well Plate, add 400 µL of the cleared lysate (NT lysate) from each well of the lysis plate used in “Part 1 step 5” of the protocol.
        2. Add 530 µL of MagMAX Binding Solution to each well in which lysate was added.

3. Add 10 µL of MagMAX Proteinase K to each well in which lysate was added.
4. Add 20 µL of MagMAX DNA/RNA Binding Beads to each well in which lysate was added. The total final volume should be 960 µL in each sample-containing well of this plate.

2. *Prepare Wash Plate 1*
    1. Add 1 mL of MagMAX Wash Buffer to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher 96 Deep Well Plate- *MagMAX Bead Binding Plate* wells.
3. *Prepare Wash Plate 2*
    1. Add 1 mL of 80% EtOH to a new KingFisher 96 Deep Well Plate matching the number and location of the KingFisher™ 96 Deep Well Plate- *MagMAX Bead Binding Plate* wells.
4. *Prepare Elution Plate*
    1. Add 100 µL of MagMAX Elution buffer to a new KingFisher 96 Deep Well Plate matching the number and location of the *MagMAX Bead Binding Plate* wells.
5. Prepare “Tip Plate”
    1. Insert the KingFisher 96 Deep Well Comb into a new KingFisher 96 Deep Well Plate
6. Run Extraction Script (Request file at sales@ceresnano.com)
    1. Run **MagMAX_96_Flex.bdz**
    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™ Flex

1. **NT_Microbiome_A_MagMAX_24_w_heat_Flex_10mL.bdz**
2. **MagMAX_96_Flex.bdz**