Nanotrap® Microbiome A; 10 mL Manual Protocol with Monarch Total RNA MiniPrep 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 Monarch Total RNA MiniPrep 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**
Monarch Total RNA MiniPrep Kit	NEB; Cat # T2010S
Materials/Equipment	**Vendor**
Heat Block	Southern Labware; SKUBSH200
Mini Centrifuge	Scientific Industries; SKU WZ-MF6000
DynaMag™-15 Magnet	Thermo Fisher Scientific; Cat# 12302D
DynaMag™-2 Magnet	Thermo Fisher Scientific; Cat# 12321D
15 mL Conical Centrifuge Tubes	Stellar Scientific; SKU T15-100
Tube Rotator	Stellar Scientific; SKU BS-RTMNI-2
Serological Pipettes and Controller	Fisherbrand; Cat# 13-678-11E
2mL Micro Centrifuge tubes	Stellar Scientific; SKU T20-100
Mini Vortex Mixer	Scientific Industries; SKU SI-236
General Reagents	**Vendor**
Ethanol	Decon™ Laboratories Decon Labs; # 3916EA
Molecular Biological Grade Water	Corning; Cat# 46-000-CM

[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 Monarch Manual Procedure-Part 1**
    1. Invert the environmental water sample 5 times to mix. Then, let it sit for 45 seconds at room temperature. (No need to wait for sample to reach room temperature before processing)
    2. Add 10 mL of environmental water sample into a clean 15 mL conical tube.
    3. Add 100 µL of Nanotrap Enhancement Reagent 2 (ER2) to the sample and then invert 2 times to mix it.
    4. Add 150 µL of Nanotrap Microbiome A Particles to the sample and then invert 2 times to mix the particles.
    5. Incubate samples with Nanotrap particles at room temperature for 10 minutes.

        Note: Invert every 5 minutes or use a rotator.
    6. Place the tube on a DynaMag-15 magnetic rack to separate the Nanotrap particles from the sample for 5 minutes.
    7. Using a serological pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: Can use a P-1000 or P-200 pipette to remove any remaining supernatant from the sample (be careful to not lose any Nanotrap particles when removing supernatant).
    8. Add 1 mL of molecular grade water to the tube and re-suspend the Nanotrap particle pellet by pipetting on the walls of the conical tube, gently re-suspend until all Nanotrap particles have been completely collected.
    9. Transfer the Nanotrap particle suspension to a new 2 mL microcentrifuge tube.
    10. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.
    11. Using a P-1000 pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.
    12. Add 500 µL of RNA Lysis Buffer to Nanotrap particle pellet, pipette up and down until Nanotrap particles are resuspended completely.
    13. Close the tube lid, incubate the samples on a heating block at 95°C for 10 minutes.
    14. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.

Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.

15. Transfer supernatant/lysate to a new 2 mL collection tube and discard the Nanotrap particles pellet.
16. Sample is now ready for Part 2.

2. Nanotrap Microbiome A Monarch Manual Procedure-Part 2

All centrifugation steps should be performed at 16,000 x g.

1. Transfer up to 500 µl of the sample/lysate from Part 1 to a gDNA Removal Column (light blue) fitted with a collection tube. For sample identification, label collection tubes, as gDNA removal columns will be discarded after spinning.
2. Spin for 30 seconds to remove most of the gDNA. SAVE THE FLOW-THROUGH (RNA partitions here). Discard the gDNA removal column.
3. Add 500 µl of ethanol (≥ 95%) to the flow-through and mix thoroughly by pipetting. Do not vortex.
4. Transfer mixture to an RNA Purification Column (dark blue) fitted with a collection tube. Spin for 30 seconds. Discard flow-through. If further gDNA removal is essential for downstream applications, proceed to on-column DNase I treatment, Step 5. If not, proceed to Step 6.
5. Optional: On-column DNase I treatment for enzymatic removal of residual gDNA
    1. Add 500 µl RNA Wash Buffer and spin for 30 seconds. Discard flow-through. This ensures all salts are removed prior to the addition of DNase I.
    2. In an RNase-free microfuge tube (not included), combine 5 µl DNase I with 75 µl DNase I Reaction Buffer and pipet mixture directly to the top of the matrix.
    3. Incubate for 15 minutes at room temperature. Proceed to Step 6.
6. Add 500 µl RNA Priming Buffer and spin for 30 seconds. Discard flow-through.
7. Add 500 µl RNA Wash Buffer and spin for 30 seconds. Discard flow-through.
8. Add another 500 µl RNA Wash Buffer and spin for 2 MINUTES. Transfer column to an RNase-free microfuge tube (not included). Use care to ensure the tip of the column does not contact the flow-through. If in doubt, re-spin for 1 minute to ensure no ethanol is carried over.
9. Add 100 µl Nuclease-free Water directly to the center of column matrix and spin for 30 seconds.
10. The sample is ready for downstream analysis or can be stored at -80ºC.

Note: Multiple freeze-thaw cycles may cause degradation.