

Manual Nanotrap[®] Wastewater Protocol using the New England Biolabs[®] Monarch[®] Total RNA Miniprep Kit

Objective: This protocol uses Nanotrap[®] Magnetic Virus Particles and Nanotrap[®] Enhancement Reagent 2 to capture and concentrate viruses in wastewater samples. It is designed for viral capture from 10 mL samples of wastewater.

Sample	
Wastewater Sample	
Equipment	Suggested Vendor
Mini Vortex Mixer	No Suggested Vendor
Magnetic Separator for 15 mL Tubes	Invitrogen [™] DynaMag [™] -15 Magnet (Thermo Fisher [™] Cat. No. 12301D)
Magnetic Separator for 1.5 mL Tubes	Invitrogen [™] DynaMag [™] -2 Magnet (Thermo Fisher [™] Cat. No. 12321D)
Mini Centrifuge (Max 6000 RPM that fits 1.5/2mL tube size)	No Suggested Vendor
Incubator	No Suggested Vendor
Materials	Suggested Vendor
Conical Tubes 15 mL (15 mL polypropylene centrifuge tubes with caps)	No Suggested Vendor
Micropipettes and Tips	No Suggested Vendor
Microcentrifuge Tubes 1.5 mL	No Suggested Vendor
Molecular Grade Water / 1x PBS without Ca ²⁺ or Mg ²⁺ (PBS)	Corning [®] (Product Number 46-000-CV)
Reagents	Suggested Vendor
80% Ethanol	Decon [™] Laboratories (Decon Labs # 3916EA)
Nanotrap [®] Enhancement Reagent 2 (ER2) ¹	Ceres Nanosciences SKU# 10112
New England Biolabs [®] Monarch [®] Total RNA Miniprep Kit	Inclusion in Kit
Monarch [®] RNA Lysis Buffer	Yes (New England Biolabs [®] Component #: T2012-1)
Further Materials	Suggested Vendor
Nanotrap [®] Magnetic Virus Particles	Ceres Nanosciences SKU# 44202

¹ Using ER2 improves viral detection by 1-2 Ct values when used with Nanotrap[®] Magnetic Virus Particles, however, it is not a required reagent for the workflow.

Precipitate can form in ER2 if stored below room temperature. Allow ER1 to return to room temperature to dissolve the precipitate.

Procedure:**A. Capture:**

1. Invert the wastewater bottle 5 times to mix. After shaking, After inverting, place on a flat surface for 45 seconds at room temperature.
2. Pipette 10 mL of wastewater sample from the wastewater bottle into a clean 15 mL conical tube.
3. To each sample add 100 μ L of Nanotrap[®] Enhancement Reagent 2 (ER2) then invert 5 times to mix thoroughly.
 - a. Note: This step is optional.
4. Add 150 μ L of Nanotrap[®] Magnetic Virus Particles to the sample. Put the cap onto the tube and invert 5 times to mix the particles.
5. Incubate samples with Nanotrap[®] Magnetic Virus Particles at room temperature for 10 minutes with constant rotation.
6. Use the 15 mL magnetic rack that is compatible with the 15 mL tube to separate the Nanotrap[®] Magnetic Virus Particles from the sample. Separation time is 5 minutes.
7. Using a pipette, discard the supernatant carefully without disturbing the Nanotrap[®] Magnetic Virus Particles pellet.
 - a. If the pellet is disturbed, repeat step 6.
8. Add 1 mL of molecular grade water to the tube and re-suspend the Nanotrap[®] Magnetic Virus Particles pellet using a pipette.
9. Transfer the Nanotrap[®] Magnetic Virus Particles and the molecular grade water to a clean 1.5 mL microcentrifuge tube.
10. Use the 2 mL magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap[®] Magnetic Virus Particles from the sample. Separation time is 2 minutes.
11. Using a pipette, discard the supernatant carefully without disturbing the pellet.
12. If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.

B. Extraction:

13. Add 300 μ L of Monarch[®] RNA Lysis Buffer to the sample and pipette up and down to resuspend the pellet.
14. Incubate for 10 minutes at room temperature.
15. Use a magnetic rack that is compatible with the 1.5 mL tube to separate the Nanotrap[®] Magnetic Virus Particles from the sample.
16. Collect the supernatant in a clean 1.5 mL microcentrifuge tube and discard the pellet using a pipette.

C. RNA Isolation/PCR Reaction Preparation:

17. Complete purification of viral RNA using the Monarch[®] Total RNA Miniprep Kit per the manufacturer's instructions (begin at Part 2, pg. 12).
18. Sample is ready for downstream assay.

Attachments: 1. NEB Monarch[®] Total RNA Miniprep Instruction Manual, V3.0 April 2020.