Automated Nanotrap® Wastewater Protocol using ZymoBIOMICS® MagBead DNA/RNA Kit

Objective: This protocol uses Nanotrap® Magnetic Virus Particles and Nanotrap® Enhancement Reagent 2 to capture and concentrate viruses in wastewater samples. It is optimized for viral capture from 10 mL samples of wastewater and is compatible with ZymoBIOMICS® MagBead DNA/RNA Kit. This method has been verified with SARS-CoV-2 viral samples.

Materials and equipment:

1. Wastewater sample
2. Nanotrap® Enhancement Reagent 2 (ER2) (Ceres Nanosciences SKU 10112)
    a. Using ER2 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER2 in this protocol if it is not available.
3. Nanotrap® Magnetic Virus Particles (Ceres Nanosciences SKU 44202)
4. ZymoBIOMICS® MagBead DNA/RNA Kit (Zymo Cat# R2135)
    a. DNA/RNA Lysis Buffer
    b. MagBinding Beads
    c. Wash Buffer 1
    d. Wash Buffer 2
    e. DNAse/RNAse Free Water
5. Thermo Scientific KingFisher™ Apex
6. Molecular Grade Ethanol
7. 3-24 well-KF Deep Well Plates
8. 1-24 well-KF Deep Well Comb
9. 4-96 well-KF Deep Well Plates
10. 1-96 well-KF Plate (200 µL)
11. 1-96 well-KF Deep Well Comb

Procedure:

Capture and Concentrate Virus using Nanotrap® Particles

1. **Ceres Nanotrap KF Procedure - Part 1**
    a. *Prepare Sample Plates*
        i. Add 4,875 µL of wastewater sample from wastewater bottle to one well (one well per sample) of a new KingFisher™ 24 Well Deep Well Plate.
        ii. Add another 4,875 µL of each sample to the same well on a second KingFisher™ 24 Well Deep Well Plate. 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.
        iii. Add 50 µL of Nanotrap® Enhancement Reagent 2 (ER2) to each wastewater sample on the two KingFisher 24 Well Deep Well sample plates.
            1. Using ER2 improves viral detection by 1-2 Ct values when used with Nanotrap® Magnetic Virus Particles, but it is ok to skip addition of ER2 and proceed to the next step.
        iv. Add 75 µL of Nanotrap® Magnetic Virus Particles to each wastewater sample on the two KingFisher 24 Well Deep Well sample plates.

v. Insert the comb into one of the sample plates. This will be "Sample Plate 1" while the other plate will be "Sample Plate 2".

b. Prepare Lysis Plate

i. Add 400 µL of Zymo Lysis Buffer to the third Kingfisher™ 24 Well Deep Well Plate matching the number and location of the "Sample Plate" wells.

c. *Run NT KingFisher™ Protocol (See attached file)*

i. Follow the on-screen instructions loading the previously prepared plates at the appropriate time.

d. Once the protocol is completed, the KingFisher™ plate to which a lysis solution was added will contain lysate that is ready to be run on the KingFisher™ Zymo MagBead extraction protocol.

2. ZymoBIOMICS MagBead KF Extraction Procedure -Part 2

a. Prepare Wash Plate 1

i. 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.

b. Prepare Wash Plate 2

i. 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.

c. Prepare Wash Plate 3

i. Add 500 µL of 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.

d. Prepare Wash Plate 4

i. Add 500 µL of 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.

e. Prepare Elution Plate

i. Add 50 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.

f. Prepare Zymo Bead Binding Plate

i. 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.

ii. Add 400 µL of Ethanol to each well in which lysate was added.

iii. Vortex the Zymo MagBinding Beads thoroughly and add 30 µL to each well.

iv. Insert the KingFisher™ 96 Deep Well Comb into the bead binding plate.

g. Run Zymo MagBead KingFisher™ Protocol (See attached file)

i. 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 will contain purified viral RNA that is ready to be loaded onto a PCR plate.

Attachments: 2

KingFisher™ Apex

1. *KF-007-WW-Nanotrap-24.kfx*
2. *KF-007-WW-Zymo-96.kfx*