Mar 04, 2024 Version 2

# W-2 WATER PROCESSING V.2

DOI
dx.doi.org/10.17504/protocols.io.ewov1opwplr2/v2

REDI-NET Consortium[1]
[1]University of Notre Dame

Remote Emerging Disea...

REDI-NET Consortium
University of Notre Dame, Naval Medical Research Center, Wal...





**DOI: https://dx.doi.org/10.17504/protocols.io.ewov1opwplr2/v2**

**Protocol Citation:** REDI-NET Consortium 2024. W-2 WATER PROCESSING . **protocols.io** **https://dx.doi.org/10.17504/protocols.io.ewov1opwplr2/v2**Version created by **REDI-NET Consortium**

**Protocol status:** Working
**We use this protocol and it's working**

**Created:** February 22, 2024

**Last Modified:** March 15, 2024

**Protocol Integer ID:** 95610

**Keywords:** total nucleic acid extraction from water sample, procedures for total nucleic acid extraction, total nucleic acid extraction, extraction from water sample, water sample, pathogen detection, total nucleic acid, extraction, disease surveillance, laboratory, collaborative laboratory network, downstream library preparation

**Funders Acknowledgements:**
**USAMRAA**
Grant ID: W81XWH-21-C-0001
**USAMRAA**
Grant ID: W81XWH-22-C-0093

## Abstract

### OBJECTIVE

To outline procedures for total nucleic acid extraction from water samples.

### SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on procedures for total nucleic acid extraction from water samples to allow downstream library preparation and sequencing for pathogen detection.

## Guidelines

### APPENDIX 3. Preparation of 40% PEG-8000 Solution

| | A | B |
|---|---|---|
| | PEG-8000 | 400 g |
| | NaCl | 70 g |
| | Add 1x PBS to final volume | 1 L |

The final concentrations: 40% PEG-8000 and [M] 1.2 Molarity (M) NaCl.

Procedure:

1. Add stir bar, PEG-8000 and NaCl into an empty 1L sterile bottle (plastic or glass, autoclavable).
2. Add sterile 1xPBS to final volume 1L.
3. Autoclave the whole bottle with lid at 121 °C for 00:30:00. After autoclave, place the hot solution on a stirring hot plate and stir the solution until it cools down to Room temperature.
4. If autoclave is not available, stir the solution on a stirring hot plate until the crystals are fully dissolved and filter the solution through 0.45 µm Corning Disposable Vacuum Filter/Storage Systems.
5. Store the 40% PEG-8000 solution at 4 °C.

### APPENDIX 4. Measuring Spoon for 0.1 mm Beating Beads

The spoon (Next Advance, MSP01-RNA) is used for 0.1 mm beating beads measurement. The step is described on step 40 the preparation before sample homogenization. One spoon equals to 100 µL.

**APPENDIX 5. DNA and RNA Measurement using QUBIT FLUOROMETER 4.0**

DNA quantification:

According to the volume of sample used, add the 1xHS dsDNA Qubit Assay for a final volume of 200 µL (i.e., if using 3 µL of sample, add 197 µL of 1x HS dsDNA Qubit Assay.

RNA Quantification:

1. In a new microcentrifuge tube/falcon tube (depending on the number of samples processed), prepare a working solution of the Qubit HS RNA Assay:

| Reagents | Volume/sample | Volume for n+1 sample |
|---|---|---|
| Qubit RNA HS Assay buffer | 199 µL | .... µL |
| Qubit RNA HS Assay Dye | 1 µL | .... µL |

2. In a new 0.6 mL tube, mix 197 µL of Qubit HS RNA Assay working solution and 3 µL of the sample. Incubate for 00:01:00 at Room temperature before reading.

**APPENDIX 6. Expected Outcomes**

| | | | | | |
|---|---|---|---|---|---|

| | Sample | Amount | Sample condition | Elution volume | DNA conc. (ng/ul) | RNA conc. (ng/ul) |
|---|---|---|---|---|---|---|
| | Tick | 1 unfed adult or 10 nymphs | Frozen/live | 75 | 20 - 30 | 10 - 20 |
| | Leech | 50 ul/ 3×3 mm/ 1 swab | Blood meal/ tissue/ swab | 75 | 5 - 100 | 5 - 100 |
| | Soil | 0.25 - 0.3 g | Frozen/Fresh | 75 | <0.025 - 20 | <0.01 - 20 |
| | Water | 750 ml | Half of the membrane | 75 | <0.025 - 20 | <0.01 - 20 |

1. **RESPONSIBLE PERSON**

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

Note

All study procedures must be conducted in compliance with national and local policies for prevention and control of COVID-19 infection.

**DATA MANAGEMENT**

Document the following information in **REDI-NET DCS W-2 Water Sample Processing** including:

- Date of water filtration, Personnel-ID, and Lab-ID
- Date of extraction, Personnel-ID, and Lab-ID
- Project-ID and associated project code
- Sample-ID
- Volume of water sample used (in ml)
- Number of membrane (10, 5, 0.45 µm) used to complete the water filtration.
- Number of half membrane subjected to the extraction.
- Extraction kit and extraction instrument
- Volume of elution of total nucleic acids
- Well-ID
- Concentration of total nucleic acids in ng/µL
- Personnel signature
- Any comments during the water filtration process or the TNA extraction

Label samples according to **REDI-NET SOP DE Data Entry**.

Water sample number is defined as follows:

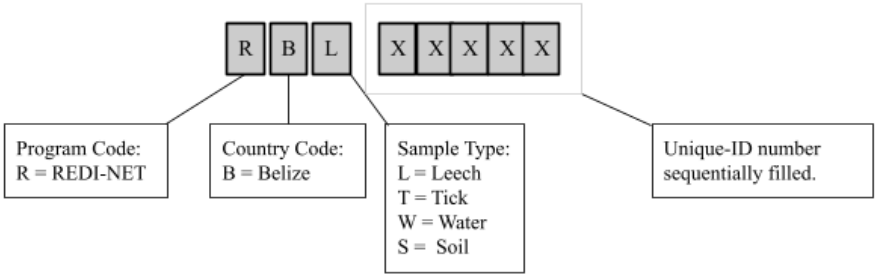
**RBTXXXXX**

Where,

**R**= Stands for the program name, REDI-NET
**B**= Stands for the country where sample collection was performed
**T**= Stands for the type of sample (i.e., T = ticks, W =water, L = leech, and S = sediment)
**XXXXX**= Stands for the unique individual sample-ID, sequentially numbered

All metadata associated with the sample that is gathered through the mobile app (in both field and lab) will be related using the unique sample-ID throughout the database.

In addition, color-coded barcode labels should be used, to enable easy recognition of sample types (example, Black=tick, Blue=water, Red= leech, and Brown=sediment).




## MAINTENANCE OF EQUIPMENT

### Caution on RNA handling:

1. RNases are very stable and difficult to inactivate and only minute amounts are sufficient to destroy RNA.
2. Care should be taken to avoid inadvertently introducing RNases into the samples during or after the purification procedure.
3. Sample handling and extraction should be performed under an extraction hood and respecting Good Laboratory Practices.
4. Use filter tips all the time.

### Cleaning filter compartments

1. Wipe magnetic filter funnel with 70% ethanol between each sample. If dirt accumulated, clean the unit with soapy water then completely rinse off the soap with deionized water. Magnetic filter funnel and 1L glass bottle are autoclavable (as well as tubing if it was made by silicon); autoclave them after filtering 2-3 batches of

water samples. If autoclave is not available, immerse the compartments in 1% bleach for 00:15:00 then rinse off the bleach and air dry the whole unit. Make sure the water inlet tube is completely dried before processing a new batch filtration. _

**Storage of the buffers from IndiMag pathogen kit**

1. Proteinase K is stable for at least 1 year after delivery when stored at Room temperature ( 15 °C — 25 °C ). To store for more than 1 year or if ambient temperature often exceeds 25 °C , storage at 2 °C — 8 °C is recommended. Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates.
2. Precipitates may form after storage at low temperature or prolonged storage. To dissolve precipitate, incubate Buffer VXL or ACB for 00:30:00 at 37 °C , with occasional shaking.
3. Reconstituted Buffer AW1 can be stored at Room temperature ( 15 °C — 25 °C ) for up to 1 year. Mix well after adding Ethanol.
4. Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases. Follow general precautions for working with RNA, such as frequent change of gloves and keeping tubes closed whenever possible.

**QUALITY CONTROL**

1. This SOP is reviewed by the applicable supervisor annually or as required in order to maintain its relevance.

# Materials

## UIPMENT AND MATERIALS

Note

*If product number is listed, please ensure use of this or equivalent product.*

| | A | B | C |
|---|---|---|---|
| | **Equipment** | **Mfg / Product #** | **NSN** |
| | KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head or KingFisher™ Duo Prime Magnetic Particle Processor | ThermoFisher, 5400630 or ThermoFisher, 5400110 | Not found |
| | Bullet Blender 24 Gold | Next Advance, BB24-AU | Not found |
| | Adjustable micropipettes | Locally sourced | N/A |
| | Multi-channel micropipettes | Locally sourced | N/A |
| | Sartorius Microsart™ Maxi Vacuum Pumps | Fisher Scientific, 14-555-788 or equivalent | Not found |
| | Vortex | Locally sourced | N/A |
| | Fisherbrand Reusable Glass Media Bottles with Cap, autoclavable | Fisher Scientific, FB8001000 or equivalent | Not found |
| | DWK Life Sciences DURAN™ Screw Cap GL 45 with 2-hose connector | Fisher Scientific, 05-719-310 | |
| | Magnetic Filter Funnel, 500 mL, 47mm | Fisher Scientific, 50-206-3019 | Not found |
| | Fisherbrand™ Heavy-Duty/Utility Funnels | | |
| | Tube centrifuge | Locally sourced | N/A |
| | Plate centrifuge | Locally sourced | N/A |
| | Qubit 4 Fluorometer | ThermoFisher, Q33238 | Not found |
| | Mini Block Heater | VWR, 10818-597 or locally sourced | Not found |
| | Autoclave | Locally sourced | N/A |
| | Stir bar | Locally sourced | N/A |

| | A | B | C |
|---|---|---|---|
| | RT2 Basic Hotplate Stirrer | ThermoFisher, 88880004 or locally sourced | Not found |

| | A | B | C | D |
|---|---|---|---|---|
| | **Material** | **Description** | **Mfg / Product #** | **NSN** |
| | Water Samples | Collected using SOP W-1; stored in 250 ml sterile bottle at 4°C for processing the same day or at -80°C or -20°C for long-term storage | From REDI-NET Program | N/A |
| | Sterile 1x PBS as negative control | 180 mL, sterile. | Thermo Fisher, 10010023 | Not found |
| | Sterile 1x PBS spike-in positive control | 180 ml sterile 1x PBS, spike in 37.5 µl of ZymoBIOMICS Microbial Community Standard and 100 µl HIV standard, 100 µl EBV standard. | Thermo Fisher, 10010023 | Not found |
| | ZymoBIOMICS Microbial Community Standard Material | For positive controls | Zymo Research, D6300 | Not found |
| | AcroMetrix HIV-1 Controls | For TNA extraction positive control; BSL-2 | ThermoFisher, CLS430320-12EA | Not found |
| | Dengue virus type 1 (DENV-1) positive control | For TNA extraction positive control | ATCC, VR-1856 | Not found |
| | Fisherbrand™ Heavy-Duty/Utility Funnels | 410 mL | Fisher Scientific, 10-500-9 | |
| | Gauze | Sterile, 4 × 4 inches, 8-ply | Fisher Scientific, 10-000-684 or equivalent | Not found |
| | Fisherbrand Reusable Glass Media Bottles | 1L, with Cap, autoclavable | Fisher Scientific, FB8001000 or equivalent | Not found |
| | DWK Life Sciences DURA Screw Cap | GL45 thread with 2-hose connector | Fisher Scientific, 05-719-310 | |
| | Thermo Scientific Nalgene 50 Platinum-Cured Silicone Tubing | 6.4 mm inner diameter, 50 ft. | Fisher Scientific, 14-176-332E or equivalent | |

| | A | B | C | D |
|---|---|---|---|---|
| | Magnetic Filter Funnel | 500 mL, fits 47mm filter (consumable) | Fisher Scientific, 50-206-3019 | Not found |
| | Nylon Membrane Filter | 30.0 µm Pore Size, 47 mm, 100/pack (consumable) | Millipore Sigma, NY3004700 | |
| | Polypropylene Membrane Filter | 10.0 µm Pore Size, 47 mm, 100/pack (consumable) | Millipore Sigma, AN1H04700 | |
| | MCE Membrane Filter | 5.0 µm Pore Size, 47 mm, 100/pk (consumable) | Millipore Sigma, SMWP04700 | |
| | Fisher brand Sterile Sampling Bags with Flat-Wire Closures | Clear, 42 oz/1190 ml, 3.5 mil thickness (consumable) | Fisher Scientific, 14-955-188 or equivalent | Not found |
| | Fisherbrand Sterile Sampling Bags with Flat-Wire Closures | Clear, 18 oz. /540 ml, 3 mil thickness (consumable) | Fisher Scientific, 14-955-186 or equivalent | Not found |
| | Beaker | 1L | Fisher Scientific, 02-591-32 or equivalent | Not found |
| | Easy Grip Polystyrene Storage Bottles | 1L (consumable) | Corning, 430518 or equivalent | Not found |
| | Easy Grip Polystyrene Storage Bottles | 250 ml (consumable) | Corning, 430281 or equivalent | Not found |
| | Cardinal Health™ Medi-Vac™ Guardian™ Suction Canisters | 3L (consumable) | Fisher Scientific, 19-162-321 or equivalent | Not found |
| | IndiMag Pathogen Kit | w/o plastics, 384 reactions | Indical Bioscience, SP947257 | Not found |
| | Buffer ATL | 200 mL, Tissue Lysis Buffer | Qiagen, 19076 | Not found |
| | Reagent DX | 1 mL, Antifoaming Reagent | Qiagen, 19088 | Not found |
| | Measuring Spoon 100µL | RNase Free, pack of 10 | Next Advance, MSP01-RNA | Not found |
| | Thermo Scientific Screw Cap Micro Tubes | 1.5 mL Screw Cap Tube, NonKnurl, NonSkirted, Natural, E-Beam Sterile tube w/ attached cap | Fisher Scientific, 14-755-208 | Not found |

| | A | B | C | D |
|---|---|---|---|---|
| | Stainless Steel UFO Beads | 3.5 mm, RNase free | Next Advance, SSUFO35-RNA | Not found |
| | Zirconium oxidase beads | 0.1 mm, 400 g | Fisher Scientific, 50-154-2950 | Not found |
| | KingFisher™ Deepwell 96 Plate | (consumable) | ThermoFisher, 95040450 | Not found |
| | KingFisher™ 96 KF microplate | KingFisher Flex ONLY (consumable) | ThermoFisher, 97002540 | Not found |
| | KingFisher™ 96 tip comb for DW Magnets | KingFisher Flex ONLY (consumable) | ThermoFisher, 97002534 | Not found |
| | KingFisher™ Duo Prime 12-tip comb | KingFisher Duo Prime ONLY (consumable) | ThermoFisher, 97003500 | Not found |
| | Elution Strip | KingFisher Duo Prime ONLY (consumable) | ThermoFisher, 97003520 | Not found |
| | KingFisher™ Duo Cap for Elution Strip | KingFisher Duo Prime ONLY (consumable) | ThermoFisher, 97003540 | Not found |
| | MicroAmp™ Clear Adhesive Film | KingFisher (consumable) | ThermoFisher, 4306311 | Not found |
| | Nonstick, RNase-Free Microfuge Tubes | 1.5 mL (consumable) | ThermoFisher, AM12450 | Not found |
| | Nonstick, RNase-Free Microfuge Tubes | 2.0 mL (consumable) | ThermoFisher, AM12475 | Not found |
| | RNaseZap™ RNase Decontamination Solution | To remove RNase from working area (consumable) | ThermoFisher, AM9780 | Not found |
| | PEG-8000 | Poly(ethylene glycol) BioUltra, 8,000 (consumable) | Millipore Sigma, 89510-1KG-F | Not found |
| | VacuCap 90 Vacuum Filtration Devices | 0.2 µm, 90 mm, gamma-irradiated, for PEG-8000 preparation(consumable) | PALL, TA4622 | Not found |
| | NaCl | For PEG-8000 buffer preparation | Sigma Aldrich, S9888-1KG | Not found |
| | Qubit™ 1X dsDNA HS Assay Kit | (consumable) | ThermoFisher, Q33230 | Not found |
| | Qubit™ RNA HS Assay Kit | (consumable) | ThermoFisher, Q32852 | Not found |

| | A | B | C | D |
|---|---|---|---|---|
| | Qubit Assay tubes | For Qubit DNA/RNA measurement (consumable) | Thermo Fisher, Q32856 | Not found |
| | Ethanol | 96-100%, molecular biology grade (consumable) | Locally Sourced | N/A |
| | Isopropanol | 100%, molecular biology grade (consumable) | Locally Sourced | N/A |
| | Forceps | Stainless, sterile (consumable) | PALL, 51147 or equivalent | Not found |
| | Tubing | Plastic (consumable) | Locally sourced | N/A |
| | Razor blades | (consumable) | Fisher Scientific, 12-640 or equivalent | Not found |
| | Petri dishes | 60 mm disposable (consumable) | Fisher Scientific, FB0875713A or equivalent | 6640-00-051-9495 |
| | Wire racks | (consumable) | Fisher Scientific, FB147916A or equivalent | Not found |
| | Excelta Medical-Grade Scissors | For filter membrane cutting | Fisher Scientific, 17-456-005 or equivalent | Not found |
| | Parafilm M | Used for petri dish sealing (consumable) | Fisher Scientific, 13-374-12 or equivalent | 6640-01-185-3289 |
| | Kimwipes | To dry material | Locally sourced | 6515-01-509-2474 |
| | Conical centrifuge tubes | 50 mL (consumable) | Fisher Scientific, 14-432-22 or equivalent | 664027047 7303 |
| | DNA/RNA Shield Reagent | 50 ml | Zymo Research, R1100-50 | Not found |
| | Conical centrifuge tubes | 15 mL (consumable) | Fisher Scientific, 14-959-53A or equivalent | 664027047 7300 |
| | Nuclease-free water | (consumable) | Locally sourced | N/A |
| | Dry ice | To maintain cold chain during sample handling (consumable) | Locally sourced | N/A |

| | A | B | C | D |
|---|---|---|---|---|
| | Data Sheets | REDI-NET DCS SP-1 Sample Processing Form | REDI-NET Data Portal | N/A |

Equipment

RT2 Basic Hotplate Stirrer
NAME

Hot Plates & Stirrers
TYPE

Thermo Scientific™
BRAND

88880004
SKU

https://www.thermofisher.com/order/catalog/product/88880004
LINK

Equipment

Mini Block Heater, Greiner Bio-One
NAME

Mini Block Heater
TYPE

Greiner Bio-One
BRAND

10818-597
SKU

https://us.vwr.com/store/product?keyword=10818-597
LINK

Equipment

Qubit 4

NAME

Fluorometer

TYPE

Invitrogen

BRAND

Q33238

SKU

https://www.thermofisher.com/order/catalog/product/Q33238

LINK

Equipment

Cytiva Magnetic Filter Funnel, 500 mL, 47 mm

NAME

Magnetic Filter Funnel

TYPE

Fisher Scientific

BRAND

50-206-3019

SKU

https://www.fishersci.com/shop/products/fltr-fnl-mag-housing-500ml/502063019

LINK

Equipment

DWK Life Sciences DURAN™ Screw Cap GL 45 with 2-hose connector

NAME

Fisher Scientific

BRAND

05-719-310

SKU

https://www.fishersci.com/shop/products/duran-screw-cap-gl-45-2-hose-connector/05719310

LINK

Equipment

Fisherbrand™ Reusable Glass Media Bottles with Cap

NAME

Reusable Glass Media Bottles

TYPE

Fisher Scientific

BRAND

FB8001000

SKU

https://www.fishersci.ca/shop/products/fisherbrand-reusable-glass-media-bottles-cap-13/fb8001000

LINK

-

Equipment

Bullet Blender 24 Gold
NAME

Bullet Blenders
TYPE

Nextadvance
BRAND

BB24AU
SKU

https://www.nextadvance.com/product/bullet-blender-24-gold/
LINK

Equipment

KingFisher Duo Prime
NAME

Automated Nucleic Acid Purification
TYPE

Thermo Scientific™
BRAND

5400110
SKU

https://www.thermofisher.com/order/catalog/product/5400110
LINK

Equipment

| | |
|---|---|
| Kingfisher Flex | NAME |
| Automated Extraction System | TYPE |
| ThermoFisher | BRAND |
| 5400630 | SKU |

PBS buffer **Thermo Fisher Scientific** **Catalog #**10010023

ZymoBIOMICS Microbial Community Standard **Zymo Research** **Catalog #**D6300

Corning tube top vacuum filtration system **Scientific Laboratory Supplies Ltd** **Catalog #**CLS430320-12EA

Equipment

| | |
|---|---|
| Fisherbrand™ Heavy-Duty/Utility Funnels | NAME |
| Fisher Scientific | BRAND |
| 10-500-9 | SKU |
| https://www.fishersci.com/shop/products/fisherbrand-heavy-duty-utility-funnels-6/105009 | LINK |

Equipment

Stoelting™ Sterile Gauze

NAME

Gauze

TYPE

Fisher Scientific

BRAND

10-000-684

SKU

https://www.fishersci.com/shop/products/sterile-gauze-7/10000684

LINK

Equipment

Thermo Scientific™ Nalgene™ 50 Platinum-Cured Silicone Tubing

NAME

Thermo Scientific™

BRAND

14-176-332E

SKU

https://www.fishersci.com/shop/products/nalgene-50-platinum-cured-silicone-tubing-1/14176332E

LINK

Equipment

| | |
|---|---|
| Nylon Net Filter | NAME |
| Hydrophilic, 30 µm, 47 mm, 100 | TYPE |
| Millipore | BRAND |
| NY3004700 | SKU |
| https://www.merckmillipore.com/IN/en/product/Nylon-Net-Filter,MM_NF-NY3004700 | LINK |

Equipment

| | |
|---|---|
| Polypropylene Prefilter | NAME |
| Hydrophobic, 10 µm, 47 mm | TYPE |
| Millipore | BRAND |
| AN1H04700 | SKU |
| https://www.merckmillipore.com/IN/en/product/Polypropylene-Prefilter-hydrophobic-10m-47mm,MM_NF-AN1H04700 | LINK |

Equipment

MF-Millipore™ Membrane Filter, 5 µm pore size

NAME

47 mm diameter, mixed cellulose esters (MCE) membrane, hydrophilic, white, 100 discs

TYPE

Millipore

BRAND

SMWP04700

SKU

https://www.merckmillipore.com/IN/en/product/MF-Millipore-Membrane-Filter-5m-pore-size,MM_NF-SMWP04700

LINK

Equipment

Whatman® Mixed Cellulose Ester filters

NAME

Membrane filters

TYPE

Millipore Sigma

BRAND

WHA7141104

SKU

https://www.sigmaaldrich.com/IN/en/product/aldrich/wha7141104

LINK

Equipment

| | |
|---|---|
| Fisherbrand™ Sterile Sampling Bags with Flat-Wire Closures | NAME |
| Fisher Scientific | BRAND |
| 14-955-188 | SKU |
| https://www.fishersci.com/shop/products/fisherbrand-sterile-sampling-bags-flat-wire-closures-16/14955188 | LINK |

Equipment

| | |
|---|---|
| Fisherbrand™ Sterile Sampling Bags | NAME |
| An economical and efficient way to collect, contain and carry samples | TYPE |
| Fisher Scientific | BRAND |
| 14-955-186 | SKU |
| https://www.fishersci.com/shop/products/fisherbrand-sterile-sampling-bags-flat-wire-closures-16/14955186 | LINK |

Equipment

Thermo Scientific™ Nalgene™ Polypropylene Griffin Low-Form Plastic Beakers

NAME

Fisher Scientific

BRAND

02-591-10G

SKU

https://www.fishersci.com/shop/products/nalgene-polypropylene-griffin-low-form-beakers/0259110G

LINK

Equipment

Cardinal Health™ Medi-Vac™ Guardian™ Suction Canisters

NAME

Suction Canisters

TYPE

Fisher Scientific

BRAND

19-162-321

SKU

https://www.fishersci.com/shop/products/cardinal-health-medi-vac-guardian-suction-canisters-2/19162321

LINK

Indical Bioscience, SP947257 **INDICAL BIOSCIENCE Catalog #**SP947257

Buffer ATL (tissue lysis buffer) **Qiagen Catalog #**19076

Reagent DX **Qiagen Catalog #**19088

Equipment

| | |
|---|---|
| Measuring Spoon | NAME |
| 100 uL RNase Free | TYPE |
| Next Advance | BRAND |
| MSP01-RNA | SKU |
| https://www.nextadvance.com/product/rnase-free-mircospoons-100-%C2%B5l/ | LINK |

Equipment

| | |
|---|---|
| Thermo Scientific™ Screw Cap Micro Tubes | NAME |
| Screw Cap Micro Tubes | TYPE |
| Fisher Scientific | BRAND |
| 14-755-208 | SKU |
| https://www.fishersci.com/shop/products/screw-cap-microcentrifuge-tubes/14755208 | LINK |

Equipment

| | |
|---|---|
| Stainless Steel UFO Beads 3.5 mm RNase Free | NAME |
| Beads | TYPE |
| Next Advance | BRAND |
| SSUFO35-RNA | SKU |
| https://www.nextadvance.com/product/ssufo35-rna-3-5-mm-rnase-free-stainless-steel-cone-beads/ | LINK |

Equipment

| | |
|---|---|
| Bertin Corp 0.1mm Zirconium oxide beads | NAME |
| Beads | TYPE |
| Fisher Scientific | BRAND |
| 50-154-2950 | SKU |
| https://www.fishersci.com/shop/products/precellys-lysing-beads-7/501542950 | LINK |

Equipment

KingFisher™ Plastics for 96 deep-well format

NAME

Automated Nucleic Acid Purification

TYPE

ThermoFisher

BRAND

95040450

SKU

https://www.thermofisher.com/order/catalog/product/95040450

LINK

Equipment

KingFisher™ Plastics for 96 standard and PCR formats Copy Icon Thermo Scientific™ KingFisher™ Plastics for 96 standard and PCR formats

NAME

Automated Nucleic Acid Purification

TYPE

Thermo Fisher Scientific

BRAND

97002540

SKU

https://www.thermofisher.com/order/catalog/product/97002540

LINK

Equipment

KingFisher™ Plastics for 96 deep-well format

NAME

Automated Nucleic Acid Purification

TYPE

Thermo Fisher Scientific

BRAND

97002534

SKU

https://www.thermofisher.com/order/catalog/product/97002534

LINK

Equipment

KingFisher™ Plastics for 96 deep-well format

NAME

Automated Nucleic Acid Purification

TYPE

ThermoFisher Scientific

BRAND

97003540

SKU

https://www.thermofisher.com/order/catalog/product/97003540

LINK

Equipment

MicroAmp™ Clear Adhesive Film — NAME

Adhesive Film — TYPE

ThermoFisher Scientific — BRAND

4306311 — SKU

https://www.thermofisher.com/order/catalog/product/4306311 — LINK

Equipment

RNase-free Microfuge Tubes — NAME

RNA Extraction — TYPE

Invitrogen — BRAND

AM12450 — SKU

https://www.thermofisher.com/order/catalog/product/AM12450 — LINK

Equipment

Nonstick, RNase-free Microfuge Tubes, 2.0 mL

NAME

Microcentrifuge tubes with a non-stick, low-binding surface

TYPE

Invitrogen

BRAND

AM12475

SKU

https://www.thermofisher.com/order/catalog/product/AM12475?SID=srch-hj-AM12475#/AM12475?SID=srch-hj-AM12475

LINK

RNaseZap® **Thermo Scientific** **Catalog #**AM9780

Polyethylenglycol (MW=8000) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**89510-1KG-F

Equipment

PALL TA4622 VacuCap 90 Vacuum Filtration Devices

NAME

0.2 µm, 90 mm (supplied with individually attached tubing for each filter device), gamma-irradiated

TYPE

Pall

BRAND

TA4622

SKU

https://www.cytivalifesciences.com/en/us/shop/lab-filtration/capsule-filters/pes-capsule-filters/vacucap-and-vacucap-pf-vacuum-filtration-devices-p-36385

LINK

Sodium Chloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S9888

Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific** **Catalog #**Q33230

Qubit RNA HS (High Sensitivity) assay **Thermo Fisher Scientific** **Catalog #**Q32852

DNA/RNA Shield **Zymo Research** **Catalog #**R1100-50

Equipment

Qubit™ Assay Tubes

NAME

Invitrogen

BRAND

Q32856

SKU

https://www.thermofisher.com/order/catalog/product/Q32856

LINK

Equipment

Forceps

NAME

PALL

BRAND

51147

SKU

https://www.dscbalances.com/products/pall-51147-forceps-stainless-steel-with-black-grips-1

LINK

Equipment

Razor Blades

NAME

Fisherbrand™

BRAND

12-640

SKU

https://www.fishersci.com/shop/products/fisherbrand-razor-blades/12640

LINK

Equipment

Fisherbrand™ Petri Dishes with Clear Lid or equivalent

NAME

Petri Dishes

TYPE

Fisherbrand

BRAND

FB0875713A

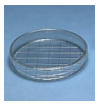
SKU

https://www.fishersci.com/shop/products/fisherbrand-petri-dishes-clear-lid-12/fb0875713a

LINK

Round, Raised Ridge, 60mm, 15mm

SPECIFICATIONS



Equipment

HDPE Coated Wire Racks

NAME

Fisherbrand™

BRAND

FB147916A

SKU

https://www.fishersci.com/shop/products/hdpe-coated-wire-racks-12/FB147916A

LINK

Equipment

| | |
|---|---|
| Laboratory Wrapping Film | NAME |
| Bemis™ | BRAND |
| 13-374-12 | SKU |
| https://www.fishersci.com/shop/products/parafilm-m-laboratory-wrapping-film-2/1337412 | LINK |

Equipment

| | |
|---|---|
| 50 mL High Clarity Conical Centrifuge Tubes | NAME |
| Falcon™ | BRAND |
| 14-432-22 | SKU |
| https://www.fishersci.com/shop/products/falcon-50ml-conical-centrifuge-tubes-2/1443222 | LINK |

## Troubleshooting

## Safety warnings

**RISK AND PERSONAL PROTECTION**

1. Caution should be taken while processing samples as some chemicals may be harmful. Please use a fume-hood when required to avoid inhaling harmful chemicals.
2. Gloves should be worn all the time when handling samples.
3. Decontaminants such as DNA/RNaZap could irritate the skin, avoid contact with skin while preparing the workbench for nucleic acid extractions.

## Before start

1. Water samples can be stored at 4 °C for 1 week, -20 °C for 1 month, and -80 °C for longer periods of time.
2. Make sure the water inlet tube and Magnetic Filter Funnel and glass bottles are properly clean and dry. If autoclave is not available, the parts in the filter system that directly contacted to the water samples needs to be fully rinsed by 10% bleach, followed by water and 70% ethanol then dry.
3. If the water samples were collected at a high temperature sampling site (≥ 25 °C ) with visible floating plants, microalgae, and sediments, use 250 mL water sample for downstream filtration, otherwise, use 750 mL water sample for filtration.
4. If water sample is frozen, fully thaw it at Room temperature then process it when it is still cold. • When water sample frozen in a plastic sample bag, wipe the bag surface with 70% ethanol to remove dusts and sanitize the surface. Place three bags of 250 mL water samples from the same sampling location in a new 1190 ml sterile sample bag, then put the bagged samples in a suitable-sized container for thawing. After samples are fully defrosted, pour the water samples into the 1190 ml outer bag, and discard the original 250 ml sample bags. Hold the whole bag in a 1 L beaker.

Note

**NOTE:** Plastic sample bags holding 250 mL water samples can leak after freeze/thaw. The 1190 mL /42 oz sterile bag can prevent sample loss/contamination.

5. Prepare 40% PEG-8000 solution for microbe aggregation. Check **Appendix 3** for the recipe.
6. Clean the work surfaces with RNaseZap, then wipe the surfaces with 70% molecular biology grade ethanol to remove additional contaminants.
7. Transfer 0.1 mm zirconium oxide beads (2 spoons, **Appendix 4**) and four 3.5 mm UFO beads to Thermo Scientific Screw Cap 1.5 mL Micro Tubes.
8. For the first time use of IndiMag pathogen kit, add 96—100% ethanol to Buffer AW1 and AW2, and add 100% Isopropanol to ACB as indicated on the bottles.
9. Buffer ATL may form precipitates upon storage. If necessary, warm to 56 °C until the precipitates have fully dissolved. Prepare buffer ATL-DX: add 100 µL Reagent DX to 15 mL Buffer ATL. If smaller amounts are needed, transfer 1.5 mL of Buffer ATL into a sterile 2 ml vial and add 10 µL Reagent DX. Mix well, after the addition of Reagent DX. After preparation, the mixture is stable for 6 months at Room temperature ( 15 °C — 25 °C ).
10. MagAttract Suspension G from the IndiMag pathogen kit needs to be vortexed thoroughly for 00:03:00 (before first use) or 00:01:00 (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.

11. Binding beads need to be vortexed thoroughly before each use.
12. Prepare a few 15 mL or 50 mL conical centrifuge tubes with nuclease-free water for preparing TNA elution in KingFisher Flex or KingFisher Duo Prime to avoid cross-contamination.

## VACUUM PUMP SET UP

1

Note

- To prevent cross contamination, nucleic acid extraction and amplification (PCR) should be performed in separate rooms.
- Processing can be done prior to freezing samples to save freezer space. Each location/site (edge/1m from edge) would account for 4 filter paper water samples for each sampling site.

Wipe the surfaces with 70% ethanol to remove contaminants.

2 Use tubing to connect a 3-liter Medi-Vac Canister with vacuum pump through the vacuum outlet on the lid. (If possible, the canister should be set up inside a biosafety cabinet).

3 Connect tubing with the 3-liter Medi-Vac Canister through the port for air-in (indicated as patient) on the lid. Close unused inlets. Turn on the pump to test the vacuum suction by feeling the airflow.

## WATER SAMPLE FILTRATION FOR CAPTURING BACTERIAL AND EUKARYOTIC TARGET

4

Note

When water sample is very dirty, filter the water sample with a sterile 8-ply gauze on a funnel using gravity to remove floating plants, mud, and microalgae, it could be done multiple times.

Assemble Magnetic Filter funnel, tubing, GL45 Screw Cap with 2-hose connector, 1L dry glass bottle (autoclaved or bleach rinsed) and the Medi-Vac Canister as Appendix 1.

Note

See Appendix 1 for the water filtration system setting. Place the Magnetic filter Funnel at a position higher than the 1L clean glass bottle, any way will do if the funnel is secure.

5 If the water samples have high turbidity, settle water at 4 °C for 01:00:00 . 1h

6 Wipe the filter holder of the magnetic funnel with 70% ethanol and let ethanol air dry.

7 Place a 30 µm filter membrane disc on the filter holder.

8 Attach the top funnel cup to the filter holder.

9 Pour the settled water sample to the magnetic funnel cup, avoid disturbing the precipitation as much as possible.

10 Turn on the pump (<15 psi) to allow the water sample to pass through the filter and be collected in the bottle (if the pump flow rate cannot be controlled, put a tube clip on the air outlet tube to control the flow rate avoiding the water splash in the bottle). Turn off the vacuum pump after the water sample runs out (If clogging happens, replace the membrane disc filter with a new one and collect all the filtrates in the same bottle).

11 Discard the 30 µm filter membrane disc.

12 Pour the filtrate to a sterile sampling bag with flat-wire closure or a clean bottle and connect the bottle back to the filtration system.

13 Place a new 10 µm filter membrane on the filter holder and filter the filtrate as step 9-10. Avoid using multiple 10 µm filter membranes to finish the filtration (the plastic bag can be reused to contain the same sample in the second round of the filtration).

14 Store the 10 µm filter membrane in a sterile 60 mm Petri dish and keep the dish On ice . Label the Petri dish with sample ID, filtration date, and membrane size.

15 Place a 5 µm filter membrane on the membrane holder then repeat step 9-10. Avoid using multiple 5 µm filter membranes to finish the filtration.

16 Store the 5 µm filter membrane in a sterile 60 mm Petri dish and keep the dish On ice . Label the Petri dish with sample ID, filtration date, and membrane size.

17 Evenly distribute 250 µL DNA/RNA Shield Reagent on the membranes in the Petri dishes, seal the Petri dishes with parafilm and store at -20 °C for short-term and -80 °C for long-term until DNA/RNA extraction.

18 Add 187 mL of PEG-8000 solution to a 750 mL water sample filtrate (or 62.5 mL PEG-8000 solution to a 250 mL filtrate, the final concentration of PEG-8000 is 8%). Mix well by shaking.

Note

If a clean 1L bottle for filtrate collection is not available, the filtrate can be transferred back to the plastic sampling bag with flat-wire closure for adding PEG-8000.

19 Rinse the magnetic funnel, water inlet tube, and 1L glass bottle with 10% bleach, then wash away the bleach with deionized water.

20 Rinse the inlet tube and 1L glass bottle with 70% ethanol, shake off the residuals and allow to air dry. Wipe dry the magnetic funnel with 70% ethanol.

21 Repeat steps from 4 to 19 for another sample.

22 To speed up the water filtration, prepare multiple sets of tubes and clean 1L bottles to avoid the waiting time for the air dry. The magnetic funnel can be used right after the 70% ethanol wipe.

23 After finishing all the sample filtration for the day, prepare a positive control for the batch: add 100 µL EBV and 50 µL DENV-1 standard into 180 mL sterile 1x PBS then add 45 mL of PEG-8000 solution.

24 Negative control for the batch of sample filtration: 180 mL sterile 1x PBS then add 45 mL of PEG-8000 solution.

25 Store PEG-8000-added samples and controls at 4 °C for more than 04:00:00 or Overnight for the next filtration round (DO NOT store the PEG-8000-added

1d 8h

samples at 4 °C more than 24:00:00 that will compromise the sample stability).

## POTENTIAL VIRAL PARTICLE COLLECTION

26 After overnight incubation with PEG-8000, water samples are ready for viral particle capturing filtration.

27 Assemble the filtration system following the steps described in steps 4-6.

28 Place a new 5 µm filter membrane on the filter holder and filter the filtrate as steps 9-10. Avoid using multiple 5 µm filter membranes to finish the filtration.

29 Store the 5 µm filter membrane in a sterile 60 mm Petri dish and keep the dish On ice. Label the Petri dish with sample ID, filtration date, and membrane size.

30 Pour the filtrate to a sterile sampling bag with flat-wire closure or a clean bottle and connect the bottle back to the filtration system.

31 Place a new 0.45 µm filter membrane on the membrane holder and filter the filtrate as steps 9-10. Avoid using multiple 0.45 µm filter membranes to finish the filtration.

32 Store the 0.45 µm filter membrane in a sterile 60 mm Petri dish and keep the dish on ice. Label the Petri dish with sample ID, filtration date, and membrane size.

33 Evenly distribute 250 µL DNA/RNA Shield Reagent on the membranes in the Petri dishes, seal the Petri dishes with parafilm and store at -20 °C for short-term and -80 °C for long-term until DNA/RNA extraction.

34 Discard the filtrate.

35 Rinse the magnetic funnel, water inlet tube, and 1L glass bottle with 10% bleach, then wash away the bleach by running under deionized water.

36 Rinse the inlet tube and 1L glass bottle with 70% ethanol, shake off the residuals, and allow to air dry.

37 Wipe dry the magnetic funnel with 70% ethanol. Repeat steps from 26 to 34 for another sample.

## SAMPLE LYSIS

38 Pre-cool the Bullet Blender by adding dry On ice into the cooling compartment and running the cooling program.

39 Clean the work surfaces with RNaseZap, then wipe the surfaces with 70% molecular biology grade ethanol to remove additional contaminants.

40 Transfer 0.1 mm zirconium oxide beads (2 spoons, Appendix 4) and four 3.5 mm UFO beads to Thermo Scientific Screw Cap 1.5 mL Micro Tubes. Each water sample needs two bead tubes. Can be prepared in advance as described in Before Start.

41 Add 500 µL of ATL-DX buffer and 135 µL VXL buffer to the Thermo Scientific Screw Cap 1.5 mL Micro Tubes containing 0.1 mm and 3.5 mm UFO beating beads.

Note

For the preparation of the ATL-DX buffer, see step "Buffer ATL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved. Prepare buffer ATL-DX: add 100 µl Reagent DX to 15 ml Buffer ATL. If smaller amounts are needed, transfer 1.5 ml of Buffer ATL into a sterile 2 ml vial and add 10 µl Reagent DX." in **before start section.**

42 Each water sample has 4 filter membranes from different filtrations (before PEG-8000 treatment: membrane of pore size 10 and 5, one of each; after PEG-8000 treatment: membrane of pore size 5 and 0.45, one of each)

43 Use 70% ethanol to wipe forceps and surgical scissors (or use new razor blade).

44 Trim the outer circle of the membrane that had no water sample flowing through off, discard the outer circle (see Figure 1).

45 Cut the membranes into 2 halves.

46 Place 4 halves of the filter membranes from different filtrations of the same water sample in a new Petri dish and store the unused half membranes in the original Petri dish at -20 °C for future use (see Figure 1).

47 Use the forceps to stack the 2 half membranes, then fold the stacked halves into a smaller sector and cut it ( smaller than 1 mm x 3 mm, see example in Figure 1) into the a tube prepared in step 40 (Suggest collecting the 2 half membranes before PEG-8000 in tube A and the 2 half membranes from after PEG-8000 treatment in tube B).

48 Add 20 µL Proteinase K from IndiMag kit and incubate the tube at 56 °C in the heat block shaker set up at 1400 rpm, 00:20:00 (if heat block shaker is not available, vortex the tube every 00:05:00 ). 25m

49 Load the sample/bead tubes in the Bullet Blender.

50 Set the speed at 12 and time at 3. Press Start.

51 Let the samples settle for 00:01:00 and then repeat step 50. 1m

STOPPING POINT: lysed samples can be stored at 4 °C Overnight .

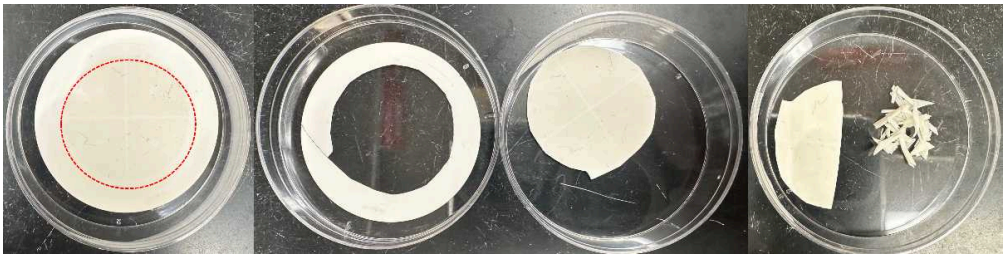


Figure 1. The examples of membrane trimming and cutting.

## INSTRUMENT SET UP (KingFisher Flex only, if using KingFisher Duo Prime, go to section "SET UP SAMPLE PLATE AND ELUTION STRIP"

52 Confirm 96 deep-well magnetic head and 96 well deep-well heat block are being used.

53 Ensure the program **IndiMag_Pathogen_KF_Flex_4wash** has been downloaded and loaded onto the KingFisher Flex instrument.

## SET UP THE PROCESSING PLATES

54 Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

> Note
>
> **DO NOT** use the elution buffer provided by the kit for TNA elution. The ingredients in the elution buffer inhibit the downstream DNA sequencing efficiency.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | Plate ID | Plate position | Plate type | Reagent | Volume per well |
| | Tip comb | 7 | Place a 96 Deep-well Tip comb in a deep-well plate | | |
| | Elution | 6 | Deep-Well | Nuclease-free water | 75 µL |
| | Wash 4 | 5 | Deep-Well | 100 % ethanol | 750 µL |
| | Wash 3 | 4 | Deep-Well | 80% ethanol | 750 µL |
| | Wash 2 | 3 | Deep-Well | Buffer AW2 | 700 µL |
| | Wash 1 | 2 | Deep-Well | Buffer AW1 | 700 µL |
| | Sample | 1 | Sample Lysate | Lysate and lysis buffer | 985 µL |

## EXTRACTION

55 Centrifuge the bead tubes with lysate from step 51 for 12000 x g, 00:05:00 . 5m

56 Transfer 425 µL supernatant without any particle carryover to the wells of the Deep-well plate. This plate becomes the Sample Plate.

57 Add 540 µL Buffer ACB, and 20 µL MagAttract Suspension G to each sample in the sample plate. For multiple samples, make a master mix with 10% overage. Invert 2m

slowly to mix the master mix, avoid foaming (can be mixed on Hula mixer for 00:02:00 ). Add 560 µL mixture to each sample.

58 Select the program **IndiMag_Pathogen_KF_Flex_4wash** on the instrument.

59 Start the run, then load the prepared plates into the positions when prompted by the instrument.

## QUANTIFICATION AND STORAGE

60 After the running protocol is completed (~ 00:35:00 ), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage. 35m

> Note
>
> The elutes from the 2 bead tubes of the same water sample can be pooled in one final tube.

61 In a 0.6 mL microcentrifuge tube, use 3 µL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer following manufacturer instructions(Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit) (see Appendix 5 and Appendix 6).

62 Proceed with sample testing following the REDI-NET SOP W-4 Water Testing or store at -20 °C for less than 2 weeks (for long-term storage the sample needs to be stored at -80 °C following the REDI-NET SOP W-3 Water Storage).

## INSTRUMENT SET UP (KingFisher Duo Prime only, if using KingFisher Flex, go to section "SET UP THE PROCESSING PLATES"

63 Confirm 12-tip magnetic head and 12 deep-well heat blocks are being used.

64 Ensure the program **IndiMag_Pathogen_KF_Duo_4wash** has been downloaded and loaded onto the KingFisher Duo Prime instrument.

## SET UP SAMPLE PLATE AND ELUTION STRIP:

65 Set up the Sample Plate according to the table below:

| | A | B | C | D |
|---|---|---|---|---|
| | **Row ID** | **Plate Row** | **Reagent** | **Volume per well** |
| | Sample row | A | Lysate and lysis buffer | 985 µL |
| | Wash 1 | B | Buffer AW1 | 700 µL |
| | Wash 2 | C | Buffer AW2 | 700 µL |
| | Wash 3 | D | 80 % ethanol | 750 µL |
| | Wash 4 | E | 100 % ethanol | 750 µL |
| | Tip Comb Wash 2 | F | 12-Tip comb | |
| | | G | Empty | |
| | | H | | |

66 Set up the Elution Strip according to the table below:

Note

***NOTE: DO NOT*** *use the elution buffer provided by the kit for TNA elution. The ingredients in the elution buffer inhibit the downstream DNA sequencing efficiency.*

| | A | B | C | D |
|---|---|---|---|---|
| | **Row ID** | **Plate Row** | **Reagent** | **Volume per well** |
| | Elution | A | Nuclease-free water | 75 µL |

## EXTRACTION

67 Centrifuge the bead tubes with lysate from step 51 for 12000 x g, 00:05:00 . 5m

68 Transfer 425 µL supernatant without any particle carryover to the wells of the Deep-well plate. This plate becomes the Sample Plate.

69 Add 540 µL Buffer ACB, and 25 µL MagAttract Suspension G to each sample in the sample plate. For multiple samples, make a master mix with 10% overage. Invert slowly to mix the master mix, avoid foaming. Add 565 µL mixture to each sample.

70 Select the program **IndiMag_Pathogen_KF_Duo_4wash** on the instrument.

71 Start the run, then load the prepared plates into position when prompted by the instrument.

72 Keep the door open while extraction. The chamber of the KingFisher Duo Prime is small. Closing the door makes the ethanol vapor restrained inside the chamber and increases the ethanol contamination.

## QUANTIFICATION AND STORAGE

73 After the running protocol is completed (~ 00:35:00 ), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage. 35m

> Note
>
> The elutes from the 2 bead tubes of the same water sample can be pooled in one final tube.

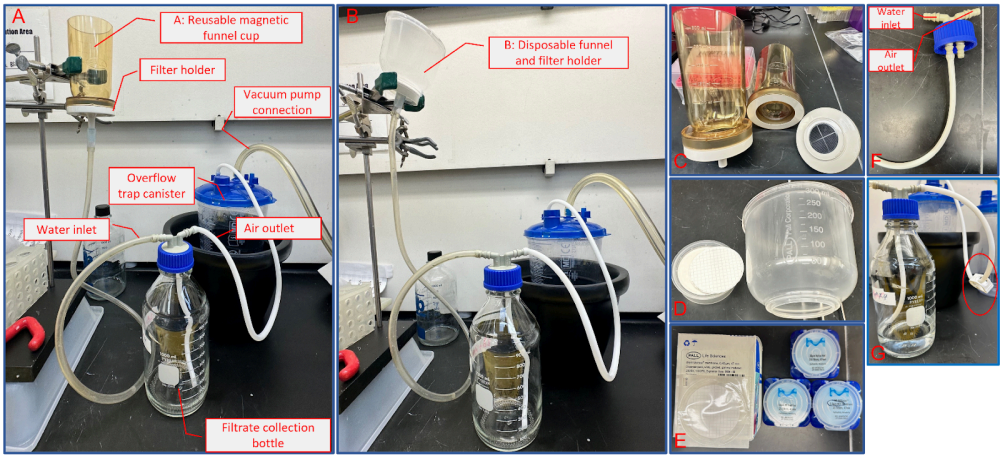
74 In a 0.6 mL microcentrifuge tube, use 3 µL total nucleic acid for DNA and RNA concentration measurement using Qubit 4 Fluorometer following manufacturer instructions (Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit). (see Appendix 5 and Appendix 6).

75 Proceed with sample testing following the REDI-NET SOP W-4 Water Testing or store at -20 °C for less than 2 weeks (for long-term storage the sample needs to be stored

at -80 °C following the REDI-NET SOP W-3 Water Storage).

## APPENDIX 1. Example of the water filtration system assembly 0m

76




Note

The reusable magnetic funnel cup (A) can be replaced by a disposable Microfunnel ST Filter funnel (B) or equivalent. Place the funnel at higher position can accelerate the filtration speed, it can be secured by any available resources as long as the setting is stable. The disposable funnel comes with a 0.45 µm membrane and secures the filter membrane by snap on the filter holding stage. The 0.45 µm can be replaced by a 30, 10, or 5 µm membrane, the support pad under the membrane needs to be kept during the filtration (D). A tube needs to be connected to the inner connector of the Screw Cap GL 45 with 2-hose connector for guide the filtrate to the bottom of the bottle to avoid the water entering the vacuum system (F). A tube clip can be secured to the air outlet tube to control the flowrate if it cannot be controlled through the vacuum pump.

## APPENDIX 2. Reference of Water Filtration Speed

77 **APPENDIX 2. Reference of Water Filtration Speed**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| | | | Before add PEG-8000 | | | After add PEG-8000 | | |
| | Sample type | Water volume | 10 µm | 5 µm | Filtration time | 5 µm | 0.45 µm | Filtration time |
| | Summer pond water with floating plants, high turbidity | 250 ml | 16 s | 1 h 24 m | 1 hr 25 m | 35 s | 4 m 14 s | 5 m 49 s |
| | Winter pond water with little floating plants | 250 ml | 10 s | 14 s | 24 s | 29 s | 3 m 07 s | 3 m 36s |

Note

The summer collected pond water was filtered through a gauze by gravity to remove all the floating plants. The water samples in this reference had been pre-filtered by 30 µm nylon membrane. A 30 µm nylon membrane can filter a 500 mL very dirty water sample within 1- 00:05:00 .

## Protocol references

1. **REDI-NET Overview Summary**
2. **REDI-NET SOP W-1 Water Collection**
3. **REDI-NET SOP W-3 Water Storage**
4. **REDI-NET SOP W-4 Water Testing**
5. **REDI-NET SOP DE Data Entry**

1. John, S.G., et al., A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environ Microbiol Rep, 2011. 3(2): p. 195-202. https://www.ncbi.nlm.nih.gov/pubmed/21572525
2. Chopyk, J., et al., Seasonal dynamics in taxonomy and function within bacterial and viral metagenomic assemblages recovered from a freshwater agricultural pond. Environ Microbiome, 2020. 15(1): p. 18. **https://www.ncbi.nlm.nih.gov/pubmed/33902740**
3. User Guide: Indical IndiMag Pathogen Kit user's manual