

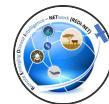


## W-2 WATER PROCESSING V.2

REDI-NET Consortium<sup>1</sup>

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Remote Emerging Disease Intelligence - NETwork (REDI-NET)



REDI-NET Consortium

University of Notre Dame, Naval Medical Research Center, Wal...

### ABSTRACT

### OBJECTIVE

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



OPEN  ACCESS

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**Protocol status:** Working

We use this protocol and it's working

Created: Feb 22, 2024

GUIDELINES

Last Modified: Mar 04, 2024

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

PROTOCOL integer ID: 95610

**Funders Acknowledgement:**

USAMRAA  
Grant ID: W81XWH-21-C-0001  
USAMRAA  
Grant ID: W81XWH-22-C-0093

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  $\text{200 } \mu\text{L}$  (i.e., if using  $\text{3 } \mu\text{L}$  of sample, add  $\text{197 } \mu\text{L}$  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	$199 \mu\text{L}$	.... $\mu\text{L}$
Qubit RNA HS Assay Dye	$1 \mu\text{L}$	.... $\mu\text{L}$

2. In a new  $0.6 \text{ mL}$  tube, mix  $197 \mu\text{L}$  of Qubit HS RNA Assay working solution and  $3 \mu\text{L}$  of the sample. Incubate for  $00:01:00$  at  $\text{Room temperature}$  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/ 3x3 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

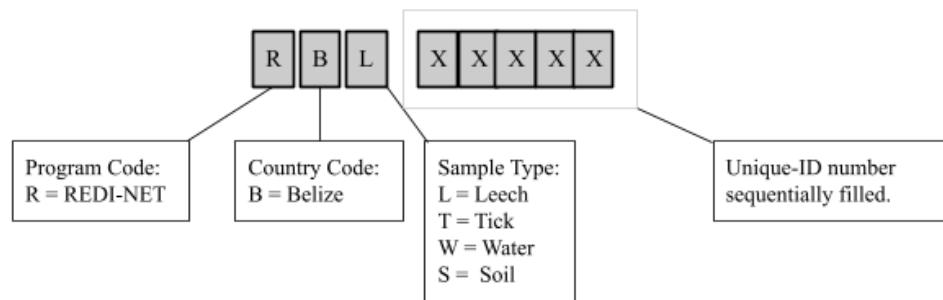
**B**= Stands for the country where sample collection was performed

**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

## EQUIPMENT 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
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 x 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	
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

A	B	C	D
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
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

A	B	C	D
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
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

A	B	C	D
Conical centrifuge tubes	50 mL (consumable)	Fisher Scientific, 14-432-22 or equivalent	6640270477 303
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	6640270477 300
Nuclease-free water	(consumable)	Locally sourced	N/A
Dry ice	To maintain cold chain during sample handling (consumable)	Locally sourced	N/A
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-<sup>LIN</sup><sub>K</sub> 6/105009](https://www.fishersci.com/shop/products/fisherbrand-heavy-duty-utility-funnels-6/105009)

## Equipment

**Stoelting™ Sterile Gauze**

NAME

Gauze

TYPE

Fisher Scientific

BRAND

10-000-684

SKU

[https://www.fishersci.com/shop/products/sterile-gauze-7/10000684<sup>LIN</sup>](https://www.fishersci.com/shop/products/sterile-gauze-7/10000684)

**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>LIN  
K**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](https://www.merckmillipore.com/IN/en/product/Nylon-Net-Filter,MM_NF-NY3004700)LIN  
K

## Equipment

### Polypropylene Prefilter

Hydrophobic, 10 µm, 47 mm

NAME

Millipore

TYPE

AN1H04700

BRAND

[https://www.merckmillipore.com/IN/en/product/Polypropylene-Prefilter-hydrophobic-10m-47mm,MM\\_NF-AN1H04700](https://www.merckmillipore.com/IN/en/product/Polypropylene-Prefilter-hydrophobic-10m-47mm,MM_NF-AN1H04700)

L  
NK

## Equipment

### MF-Millipore™ Membrane Filter, 5 µm pore size

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

NAME

TYP  
E

Millipore

BRAND

SMWP04700

SKU

[https://www.merckmillipore.com/IN/en/product/MF-Millipore-Membrane-Filter-5m-pore-size,MM\\_NF-SMWP04700](https://www.merckmillipore.com/IN/en/product/MF-Millipore-Membrane-Filter-5m-pore-size,MM_NF-SMWP04700)

LIN  
K

## Equipment

**Whatman® Mixed Cellulose Ester filters**

NAME

Membrane filters

TYPE

Millipore Sigma

BRAND

WHA7141104

SKU

<https://www.sigmaaldrich.com/IN/en/product/aldrich/wha7141104><sup>LINK</sup>

## 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><sup>LINK</sup>

## Equipment

**Fisherbrand™ Sterile Sampling Bags**

An economical and efficient way to collect, contain and carry samples

NAME

Fisher Scientific

TYPE

14-955-186

BRAND

<https://www.fishersci.com/shop/products/fisherbrand-sterile-sampling-bags-flat-wire-closures-16/14955186>

SKU

## Equipment

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

Fisher Scientific

BRAND

02-591-10G

SKU

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

## Equipment

<b>Cardinal Health™ Medi-Vac™ Guardian™ Suction Canisters</b>	NAME
Suction Canisters	TYPE
Fisher Scientific	BRAND
19-162-321	SKU
<a href="https://www.fishersci.com/shop/products/cardinal-health-medi-vac-guardian-suction-canisters-2/19162321">https://www.fishersci.com/shop/products/cardinal-health-medi-vac-guardian-suction-canisters-2/19162321</a>	LINK

-  Indical Bioscience, SP947257 INDICAL BIOSCIENCE Catalog #SP947257
-  Buffer ATL (tissue lysis buffer) Qiagen Catalog #19076
-  Reagent DX Qiagen Catalog #19088

## Equipment

<b>Measuring Spoon</b>	NAME
100 uL RNase Free	TYPE
Next Advance	BRAND
MSP01-RNA	SKU
<a href="https://www.nextadvance.com/product/rnase-free-mircospoons-100-%C2%B5l/">https://www.nextadvance.com/product/rnase-free-mircospoons-100-%C2%B5l/</a>	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** NA  
**Thermo Scientific™ KingFisher™ Plastics for 96 standard and PCR** M  
**formats** E

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><sup>LINK</sup>

## 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><sup>LINK</sup> [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), <sup>TYP</sup><sub>E</sub> gamma-irradiated

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>

<sup>LI</sup>  
<sup>N</sup>  
<sup>K</sup>

 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><sup>LINK</sup>

## Equipment

**Razor Blades**

NAME

Fisherbrand™

BRAND

12-640

SKU

<https://www.fishersci.com/shop/products/fisherbrand-razor-blades/12640><sup>LINK</sup>

## 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>LIN  
K

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>LIN  
K

## Equipment

**Laboratory Wrapping Film**

NAME

Bemis™

BRAND

13-374-12

SKU

<https://www.fishersci.com/shop/products/parafilm-m-laboratory-wrapping-film-2/1337412>

LIN  
K

## 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>

LIN  
K

## 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 INSTRUCTIONS

1. Water samples can be stored at  $\text{4 }^{\circ}\text{C}$  for 1 week,  $\text{-20 }^{\circ}\text{C}$  for 1 month, and  $\text{-80 }^{\circ}\text{C}$  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 ( $\geq \text{25 }^{\circ}\text{C}$ ) with visible floating plants, microalgae, and sediments, use  $\text{250 mL}$  water sample for downstream filtration, otherwise, use  $\text{750 mL}$  water sample for filtration.
4. If water sample is frozen, fully thaw it at  $\text{Room temperature}$  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  $\text{250 mL}$  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  $\text{250 mL}$  water samples can leak after freeze/thaw. The  $\text{1190 mL}$  /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  $\text{56 }^{\circ}\text{C}$  until the precipitates have fully dissolved. Prepare buffer ATL-DX: add  $\text{100 }\mu\text{L}$  Reagent DX to  $\text{15 mL}$  Buffer ATL. If smaller amounts are needed, transfer  $\text{1.5 mL}$  of Buffer

ATL into a sterile 2 ml vial and add  $\text{10 } \mu\text{L}$  Reagent DX. Mix well, after the addition of Reagent DX. After preparation, the mixture is stable for 6 months at  $\text{Room temperature}$  ( $15^\circ\text{C} - 25^\circ\text{C}$ ).

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  $15 \text{ mL}$  or  $50 \text{ mL}$  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 EUKAR...

4

**Note**

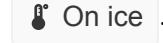
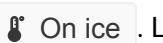
When water sample is very dirty, filter the water sample with a sterile 8-ply gauze on a funnel using gravity to remove floating plants, mud, and microalgae, it could be done multiple times.

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  . 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  . Label the Petri dish with sample ID, filtration date, and membrane size.

- 17 Evenly distribute  $\text{250 } \mu\text{L}$  DNA/RNA Shield Reagent on the membranes in the Petri dishes, seal the Petri dishes with parafilm and store at  $-20^\circ \text{C}$  for short-term and  $-80^\circ \text{C}$  for long-term until DNA/RNA extraction.
- 18 Add  $\text{187 mL}$  of PEG-8000 solution to a  $\text{750 mL}$  water sample filtrate (or  $\text{62.5 mL}$  PEG-8000 solution to a  $\text{250 mL}$  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  $\text{100 } \mu\text{L}$  EBV and  $\text{50 } \mu\text{L}$  DENV-1 standard into  $\text{180 mL}$  sterile 1x PBS then add  $\text{45 mL}$  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, 1d 8h for the next filtration round (DO NOT store the PEG-8000-added 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  $\text{500 } \mu\text{L}$  ATL-DX buffer and  $\text{135 } \mu\text{L}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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 25m set up at 1400 rpm, 00:20:00 (if heat block shaker is not available, vortex the tube every 00:05:00 ).

**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 Prim...)

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

A	B	C	D	E
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. 2m  
 For multiple samples, make a master mix with 10% overage. Invert 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 Fle...

- 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
<b>Row ID</b>	<b>Plate Row</b>	<b>Reagent</b>	<b>Volume per well</b>
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

A	B	C	D
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

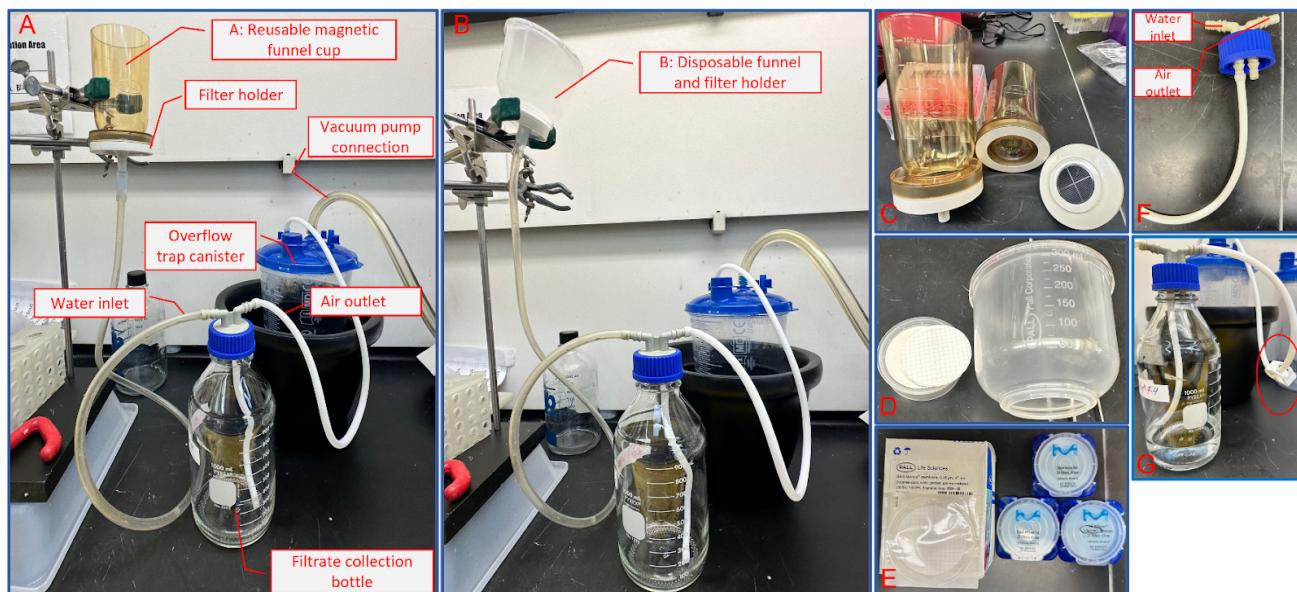
### Note

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  $\mu$ L 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

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### 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

A	B	C	D	E	F	G	H
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.