S-2 SOIL PROCESSING

REDI-NET Consortium[1]

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We use this protocol and it's working

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DISCLAIMER

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ABSTRACT

This protocol details about soil processing.

GUIDELINES

OBJECTIVE

To outline procedures for total nucleic acid extraction from soil 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 soil samples to provide materials for downstream library preparation and sequencing for pathogen detection.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

PROTOCOL integer ID:
81782

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Note

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

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.

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 (15-25°C). To store for more than 1 year or if ambient temperature often exceeds 25 °C, storage at 2-8 °C is recommended. Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates.
2. Precipitation 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 (15-25°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

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

APPENDICES

APPENDIX 1. MEASURING SPOON FOR 0.1 MM BEATING BEADS

The spoon (Next Advance, MSP01-RNA) is used for 0.1 mm beating beads measurement. One spoon equals to 100 uL.

APPENDIX 3. EXPECTED OUTCOMES

Expected result

	A	B	C	D	E	F
	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

MATERIALS

EQUIPMENT AND MATERIALS

Note

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

	A	B
	Equipment	**Mfg / Product #**
	KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head or KingFisher™ Duo Prime Magnetic Particle Processor	ThermoFisher, 5400630 or ThermoFisher, 5400110
	Bullet Blender 24 Gold	Next Advance, BB24-AU
	Qubit 4 Fluorometer	ThermoFisher, Q33238
	Adjustable micropipettes	Locally sourced
	Multi-channel micropipettes	Locally sourced

	A	B
	Vortex	Locally sourced
	Tube centrifuge	Locally sourced
	Plate centrifuge	Locally sourced
	Digital scale/balance	Locally sourced
	Thermo Heater Mixer	Locally sourced

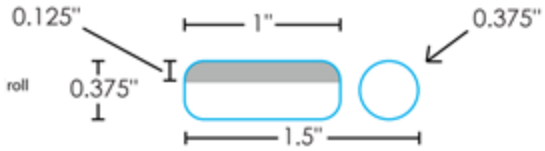
	A	B	C
	Material	**Description**	**Mfg / Product #**
	IndiMag Pathogen Kit (*MagMAX Microbiome Ultra Nucleic Acid Isolation Kit can be used instead if already procured*)	w/o plastics, 384 reactions	Indical Bioscience, SP947257
	Buffer ATL (*for IndiMag kit only*)	200 mL, Tissue Lysis Buffer	Qiagen, 19076
	Reagent DX (*for IndiMag kit only*)	1 mL, Antifoaming Reagent	Qiagen, 19088
	Measuring Spoon 100 µL	RNase Free, pack of 10. reusable	Next Advance, MSP01-RNA
	KingFisher™ Deepwell 96 Plate	KingFisher	ThermoFisher, 95040450
	KingFisher™ 96 KF microplate	KingFisher Flex *ONLY*	ThermoFisher, 97002540
	KingFisher™ 96 tip comb for DW magnets	KingFisher Flex *ONLY*	ThermoFisher, 97002534
	KingFisher™ Duo Prime 12-tip comb	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003500
	Elution Strip	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003520
	KingFisher™ Duo Cap for Elution Strip	KingFisher Duo Prime *ONLY*	ThermoFisher, 97003540
	MicroAmp™ Clear Adhesive Film	KingFisher	ThermoFisher, 4306311
	RNase-Free Microfuge Tubes	Nonstick, 1.5 mL	ThermoFisher, AM12450
	RNase-Free Microfuge Tubes	Nonstick, 2.0 mL	ThermoFisher, AM12475
	Clear RINO brand microcentrifuge tubes	1.5 mL, screw-cap	Next Advance, TUBE1R5-S
	Zirconium oxide beads	0.1 mm, 400 g	Fisher Scientific, 50-154-2950
	Qubit™ 1X dsDNA HS Assay Kit	(*consumable)*	ThermoFisher, Q33230
	Qubit™ RNA HS Assay Kit	(*consumable)*	ThermoFisher, Q32852

	A	B	C
	RNaseZap™ RNase Decontamination Solution	To remove RNase from working area	ThermoFisher, AM9780
	ZymoBIOMICS Microbial Community Standard Material	For positive controls	Zymo Research, D6300
	AcroMetrix HIV-1 Controls	For TNA extraction positive control	ThermoFisher, CLS430320-12EA
	Human gammaherpesvirus (EBV) positive control	For TNA extraction positive control	NMRC made
	Spatula	For use with samples	Locally sourced
	Ethanol	100% (molecular biology grade)	Locally sourced
	Isopropanol (*for IndiMag kit only*)	100% (molecular biology grade)	Locally sourced
	Nuclease-free Water	To elute total nucleic acids	Locally sourced
	Dry ice	To maintain cold chain during sample handling	Locally sourced
	Ice bucket	To contain the dry ice	Locally sourced
	Kimwipes	To dry material	Locally sourced
	Qubit Assay Tubes	For Qubit DNA/RNA measurement *(consumable)*	Thermo Fisher, Q32856
	Falcon tubes	15 mL and 50 mL	Locally sourced
	Data sheets	REDI-NET DCS SP-1 Sample Processing Form	REDI-NET Data Portal

APPENDIX 4. SET-UP INSTRUCTIONS FOR BARCODE PRINTING

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**
	Thermal Printer	Zebra ZD421T Desktop Dual Barcode Printer - 203 dpi	Uline, H-9581
	Thermal Transfer Ribbon	For use with Zebra thermal printer; Desktop thermal transfer ribbons - wax/resin, 4.33” x 244 (12/case)	Uline, S-18466

	A	B	C
	Cryo-labels	667 1.00" x 0.38" Cap & Wrap CryoLabel® w/0.375" Cap, Blanks, 1" Core Color bar breakdown: Grey - 31,24,25,0 Orange - 0,80,95,0 Blue - 85,50,0,0 Brown - 35,60,80,25 Yellow - 0,0,100,0	Electronic Imaging Materials, #335774-COLOR
	Handheld scanner	To scan barcode	Zebra, LS2208-SR20001R-NA
	123Scan Software	To scan barcodes	123Scan software
	Laptop or desktop computer with Google Chrome and access to the REDI-NET data portal	To connect with the handheld scanner, the thermal printer and the REDI-NET Data Portal	Locally sourced



Cryo-labels

Equipment

KingFisher™ Duo Prime Purification System NAME

Purification System TYPE

Thermo Scientific™ BRAND

5400110 SKU

https://www.thermofisher.com/order/catalog/product/5400110?SID=srch-srp-5400110 LINK

Equipment

Bullet Blender 24 Gold (1.5 mL snap and screw cap tubes, 4°C cooling)	NAME
Blender	TYPE
Next Advance	BRAND
BB24-AU	SKU
https://www.nextadvance.com/product/bullet-blender-24-gold/	LINK

Equipment

Qubit Fluorometer	NAME
Fluorometer	TYPE
Invitrogen	BRAND
Q33238	SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238	LINK

IndiMag Pathogen Kit w/o plastics (384 reactions) **INDICAL BIOSCIENCE Catalog #SP947257**

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

Reagent DX **Qiagen Catalog #19088**

Measuring Spoon 100 uL RNase Free pack of 10 **Next Advance Catalog #MSP01-RNA**

KingFisher™ Plastics for 96 deep-well format **Thermo Fisher Scientific Catalog #95040450**

KingFisher Microplate **Thermo Fisher Scientific Catalog #97002540**

KingFisher™ Flex™ Systems Consumables, KingFisher 96 tip comb for DW magnets **Thermo Fisher Catalog #97002534**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, 12-tip comb, for Microtiter 96 Deepwell plate **Thermo Fisher Catalog #97003500**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, Elution strip **Thermo Fisher Catalog #97003520**

KingFisher™ Duo and KingFisher™ Duo Prime Consumables, KingFish Duo Cap for elution strip **Thermo Fisher Catalog #97003540**

MicroAmp™ Clear Adhesive Film **Thermo Fisher Scientific Catalog #4306311**

Nonstick, RNase-free Microfuge Tubes, 1.5 mL **Thermo Fisher Catalog #AM12450**

Nonstick, RNase-free Microfuge Tubes, 2.0 mL **Thermo Fisher Catalog #AM12475**

Sterile Microcentrifuge Tube 1.5 mL (RINO®) 500/case **Next Advance Catalog #TUBE1R5-S**

Bertin Corp 0.1mm Zirconium oxide beads (450g) (qty 500) **Fisher Scientific Catalog #50-154-2950**

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

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

RNaseZap® **Thermo Scientific Catalog #AM9780**

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

Qubit™ Assay Tubes **Invitrogen - Thermo Fisher Catalog #Q32856**

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

BEFORE START

Note

NOTE:

To prevent contamination samples nucleic acid extraction and amplification (PCR) should be performed in separate rooms.

1. Pre-cool the Bullet Blender by adding dry ice into the cooling compartment and running the cooling program.
2. Clean the work surfaces with RNaseZap, then wipe the surfaces with 70% molecular biology grade ethanol to remove additional contaminants.
3. Transfer 0.1 mm zirconium oxide beads (2 spoons, Appendix 1) to Clear RINO brand 1.5 ml screw-cap microcentrifuge tubes.*
4. For the first time use of IndiMag pathogen kit, add 100% ethanol to Buffer AW1 and AW2, and add 100% isopropanol to ACB as indicated on the bottles (*Optional if using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit*).
5. 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 addition of Reagent DX. After preparation, the mixture is stable for 6 months at Room temperature (15-25°C)**
6. MagAttract Suspension G from 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.**

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, transfer **all the 0.1 mm beating beads into a new clear RINO tube brand 1.5 mL screw-cap microcentrifuge tube.*

***Optional if using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit.*

1. SAMPLE LYSIS

1 Add 320 µL of 1x PBS and 80 µL ATL-DX Buffer to the bead tubes prepared on step 3 of Before Start section under the Guidelines & Warnings tab.

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, add 800 µL of Lysis Buffer to the clear RINO 1.5mL prepared on step 3 of Before Start section.

2

Note

If soil is frozen, thaw and keep it On ice.

Use a clean spatula or forceps to weigh about 0.25 g soil sample and place it into each prepared bead tube. Record the weight.

3 Include a positive control for each batch of samples: transfer 37.5 µL ZymoBIOMICS Microbial Community Standard Material and µL EBV, and 100 µL HIV standard into a tube from step 3 of Before Start section. Add 162.5 µL 1xPBS.

4 Include a negative control for each batch of samples: a bead tube from step 3 of Before Start section with 320 µL cold sterile 1xPBS only.

5 Add dry ice into the cooling compartment of Bullet Blender and then load the all bead tubes (samples and controls).

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

7 Let the samples settle for 00:01:00 and then repeat step 6. 1m

Note

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

2. INSTRUMENT SET UP

8

Note

NOTE: KingFisher Flex only, if using KingFisher Duo Prime, Section 7

Confirm 96 deep-well magnetic heads and 96 well deep-well heat blocks are being used.

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

Note

*If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit ensure the program **MagMax_Microbiome_Soil_Flex** has been downloaded and loaded onto the instrument.*

3. SET UP THE PROCESSING PLATES

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

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	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

	A	B	C	D	E
	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	990 µL

10.1

Note

NOTE: *If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, set up the Wash, Elution, Tip Comb Plates outside the instrument according to the following table.*

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash 1 Plate	2	Deep-well	Wash Buffer	1,000 µL
Wash 2 Plate	3	Deep-well	Wash Buffer	1,000 µL
Wash 3 Plate	4	Deep-well	80% Ethanol	1,000 µL
Wash 4 Plate	5	Deep-well	80% Ethanol	1,000 µL
Elution Plate	6	Deep-well	Elution Solution	70µL
Tip Comb	7	Place a 96 Deep-well Tip Comb in a Standard deep-well Plate		

4. EXTRACTION

11 Centrifuge the bead tubes with lysate from step 7 for 12000 x g, 00:05:00. 5m

12 Add 20 µL of Proteinase K into wells (based on number of samples) of a new Deep-well plate.

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, add 40 µL of Proteinase K to each sample.

13 Transfer 270 µL supernatant without any particle carryover to the wells of the Deep-well plate containing proteinase K. This plate becomes the Sample Plate.

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, transfer as much supernatant as possible (up to 500 µL).

14 Add 135 µL Buffer VXL, 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 slowly to mix the master mix, avoid foaming (can be mixed on Hula mixer for 2 min). Add 695 µL mixture to each sample (Optional if using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit).

15 Select the program **IndiMag_Pathogen_KF_Flex_4wash** or the program **MagMAX_Microbiome_Liquid_Buccal_Flex** on the instrument according to the kit used.

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

5. BIND, WASH AND ELUTE (Only if using the MagMAX Microbi...

17 Vortex magnetic beads vigorously and for each sample, transfer 20 µL beads to 500 µL Binding Buffer. Make the master mix for multiple samples with 10% overage. Mix the master mix by inverting, then place the master mix on a rocker until use (Do not vortex).

18 When prompted (approximately 20 minutes after the start of the protocol), remove the Sample plate from the instrument.

19 Invert Binding Bead mix prepared in step 17 to mix, then add 520 µL to each sample in the Sample Plate. Remix the Binding Bead mix frequently to ensure even distribution of beads to all samples.

20 Place the Sample Plate back onto the instrument, then start the run.

6. QUANTIFICATION AND STORAGE

21 After the running protocol is completed (~35 minutes), 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.

22 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.

Note

Kits needed: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see Appendix 2 and Appendix 3)

23 Proceed with sample testing following the REDI-NET SOP S-4 Soil Testing or store at -20 °C for less than 2 weeks.

Note

For long-term storage the sample needs to be stored at -80 °C following the REDI-NET SOP S-3 Soil Storage.

7. INSTRUMENT SET UP

24

Note

NOTE: KingFisher Duo Prime only, if using KingFisher Flex, go to section 2

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

25 Ensure the program **IndiMag_Pathogen_KF_Duo_4wash** has been downloaded and loaded onto the

KingFisher Duo Prime instrument.

Note

*If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit ensure the program **MagMAX_Microbiome_Soil_Duo** has been downloaded and loaded onto the instrument.*

8. SET UP THE SAMPLE PLATE AND ELUTION STRIP

26 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	F	Tip comb	
		G	Empty	
		H		

26.1

Note

***NOTE:** If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, set up the Wash, Elution, Tip Comb Plates outside the instrument according to the following table.*

Row ID	Plate Row	Reagent	Volume per well
Sample row	A	Sample	Varies
Tip Comb	B	Tip Comb	Empty
—	C	Empty	
80% Ethanol	D	80% Ethanol	1,000 µL
80% Ethanol	E	80% Ethanol	1,000 µL
Wash Buffer	F	Wash Buffer	1,000 µL
Wash Buffer	G	Wash Buffer	1,000 µL
—	H	Empty	

27 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

27.1

Note

NOTE: *If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, set up the Elution plates outside the instrument according to the table below:*

Row ID	Plate Row	Reagent	Volume per well
Elution Solution	A	Elution Solution	70µL

9. EXTRACTION

5m

28 Centrifuge the bead tubes with lysate from Sample Lysis step 7 for 12000 x g, 00:05:00 . 5m

29 Add 20 µL of Proteinase K into wells (based on number of samples) of a new Deep-well plate.

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, add 40 µL of Proteinase K to each sample.

30 Transfer 270 µL supernatant without any particle carryover to the wells of the Deep-well plate containing proteinase K. This plate becomes the Sample Plate.

Note

If using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, transfer as much supernatant as possible (up to 500 µL).

31 Add 135 µL Buffer VXL, 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 slowly to mix the master mix, avoid foaming. Add 695 µL mixture to each sample (Optional if using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit).

32 Select the program **IndiMag_Pathogen_KF_Duo_4wash** or the program **MagMAX_Microbiome_Soil_Duo** on the instrument.

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

Note

Keep the door open while extraction is in process. 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.

10. BIND, WASH AND ELUTE(Only if using the MagMAX Microb...

34 Vortex magnetic beads vigorously and for each sample, transfer 20 µL beads to 500 µL Binding Buffer. Make master mix for multiple samples with 10% overage. Mix the master mix by inverting, then place the master mix on a rocker until use (Do not vortex).

35 When prompted (approximately 00:20:00 after the start of the protocol), remove the Sample pl 20m from the instrument.

36 Invert Binding Bead mix prepared in step 35 to mix, then add 520 µL to each sample in the Sample Plate. Remix the Binding Bead mix frequently to ensure even distribution of beads to all samples.

37 Place the Sample Plate back onto the instrument, then start the run.

11. QUANTIFICATION AND STORAGE

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

39 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.

> **Note**
>
> **Kits needed**: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see Appendix 2 and Appendix 3)

40 Proceed with sample testing following the REDI-NET SOP S-4 Soil Testing or store at -20 °C for less than 2 weeks.

> **Note**
>
> For long-term storage the sample needs to be stored at -80°C following the REDI-NET SOP S-3 Soil Storage.

APPENDIX 2. DNA and RNA Measurement using QUBIT FLUORO.. 2m

41 DNA quantification: 2m

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. Vortex for 5 - 10 seconds, then Incubate for 00:02:00 at Room temperature before reading.

42 RNA Quantification:

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

	A	B	C
	Reagents	Volume/sample	Volume for n+1 sample
	Qubit RNA HS Assay buffer	199 µL	…. µL
	Qubit RNA HS Assay Dye	1 µL	…. µL

42.2 In a new 0.6 ml tube, mix 197 µL of Qubit HS RNA Assay working solution and 3 µL of th 2m sample. Vortex for 5 - 10 seconds, then incubate for 00:02:00 at Room temperature before reading.