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Version 2

## REDI-NET F-2 FECES PROCESSING V.2

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Remote Emerging Disea...



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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 feces 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

#### 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 µL .



### **APPENDIX 3. EXPECTED OUTCOMES**

## Expected result

|  | A             | B                          | C                        | D                     | E                        | F                        |
|--|---------------|----------------------------|--------------------------|-----------------------|--------------------------|--------------------------|
|  | <b>Sample</b> | <b>Amount</b>              | <b>Sample condition</b>  | <b>Elution volume</b> | <b>DNA conc. (ng/ul)</b> | <b>RNA conc. (ng/ul)</b> |
|  | 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                                |
| 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          | w/o plastics, 384 reactions      | Indical Bioscience, SP947257 |
| Buffer ASL                    | 140 mL, Stool Lysis Buffer       | Qiagen, 19082                |
| Measuring Spoon 100 µL        | RNase Free, pack of 10. reusable | Next Advance, MSP01-RNA      |
| KingFisher™ Deepwell 96 Plate | KingFisher                       | ThermoFisher, 95040450       |



| A                                                 | B                                                                                         | C                                  |
|---------------------------------------------------|-------------------------------------------------------------------------------------------|------------------------------------|
| KingFisher™ 96 KF microplate                      | KingFisher Flex <i>ONLY</i>                                                               | ThermoFisher, 97002540             |
| KingFisher™ 96 tip comb for DW magnets            | KingFisher Flex <i>ONLY</i>                                                               | ThermoFisher, 97002534             |
| KingFisher™ Duo Prime 12-tip comb                 | KingFisher Duo Prime <i>ONLY</i>                                                          | ThermoFisher, 97003500             |
| Elution Strip                                     | KingFisher Duo Prime <i>ONLY</i>                                                          | ThermoFisher, 97003520             |
| KingFisher™ Duo Cap for Elution Strip             | KingFisher Duo Prime <i>ONLY</i>                                                          | ThermoFisher, 97003540             |
| MicroAmp™ Clear Adhesive Film                     | KingFisher                                                                                | ThermoFisher, 4306311              |
| RNase-Free Microfuge Tubes                        | Nonstick, 1.5 mL                                                                          | ThermoFisher, AM12450              |
| 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      |
| 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               |
| Qubit Assay Tubes                                 | For Qubit DNA/RNA measurement (consumable)                                                | Thermo Fisher, Q32856              |
| RNaseZap™ RNase Decontamination Solution          | To remove RNase from working area                                                         | ThermoFisher, AM9780               |
| ZymoBIOMICS Microbial Community Standard Material | For positive controls                                                                     | Zymo Research, D6300               |
| Dengue virus type 1 (DENV-1) positive control     | For TNA extraction positive control                                                       | ATCC, VR-1856 or locally resourced |
| Human gammaherpesvirus (EBV) positive control     | For TNA extraction positive control                                                       | NMRC made                          |
| Forceps                                           | For use with samples                                                                      | Locally sourced                    |
| Spatula                                           | For use with samples                                                                      | Locally sourced                    |
| Ethanol                                           | 100% (molecular biology grade)                                                            | Locally sourced                    |
| Isopropanol                                       | 100% (molecular biology grade)                                                            | Locally sourced                    |
| Nuclease-free Water                               | To elute total nucleic acids                                                              | Locally sourced                    |

|  | A            | B                                             | C                    |
|--|--------------|-----------------------------------------------|----------------------|
|  | 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      |
|  | Falcon tubes | 15 mL and 50 mL                               | Locally sourced      |
|  | Data sheets  | REDI-NET DCS SP-1 Sample Processing Form      | REDI-NET Data Portal |

Equipment

KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head

Purification System

Thermo Scientific™

5400630

<https://www.thermofisher.com/order/catalog/product/5400630>

NAME

TYPE

BRAND

SKU

LINK

Equipment

KingFisher™ Duo Prime Purification System

Purification System

Thermo Scientific™

5400110

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

NAME

TYPE

BRAND

SKU

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   |
| <a href="https://www.nextadvance.com/product/bullet-blender-24-gold/">https://www.nextadvance.com/product/bullet-blender-24-gold/</a> |  | LINK  |

## Equipment

|                                                                                                                                                   |  |       |
|---------------------------------------------------------------------------------------------------------------------------------------------------|--|-------|
| Qubit Fluorometer                                                                                                                                 |  | NAME  |
| Fluorometer                                                                                                                                       |  | TYPE  |
| Invitrogen                                                                                                                                        |  | BRAND |
| Q33238                                                                                                                                            |  | SKU   |
| <a href="https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238">https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238</a> |  | LINK  |

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

Buffer ASL (560 ml) **Qiagen Catalog #19082**

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

KingFisher<sup>®</sup>; Flex<sup>®</sup>; Systems Consumables, KingFisher Flex Microtiter Deepwell 96 plate, V-bottom **Thermo Fisher 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, KingFisher Duo Cap for elution strip **Thermo Fisher Catalog #97003540**

 MicroAmp®; Clear Adhesive Film **Thermo Fisher Catalog #4306311**

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

 Thermo Scientific™ Screw Cap Micro Tubes **Fisher Scientific Catalog #14-755-208**

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

 Qubit™ 1X dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits **Thermo Fisher Scientific Catalog #Q33230**

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

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

 RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #AM9780**

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



## 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

### **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 irconium 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 (before first use) or (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.



## 1. SAMPLE LYSIS

- 1 Add  700  $\mu\text{L}$  of ASL buffer to the bead tubes prepared in step 4 from "Before Start" section under the Guidelines & Warning tab. 
- 2 Use a clean spatula or forceps to weigh about  100 mg fecal sample (roughly 5 drops of mouse feces) and place it into each prepared bead tube (If fecal sample is frozen, thaw and keep it  On ice ). Record the weight.
- 3 Include a positive control for each batch of samples: transfer  37.5  $\mu\text{L}$  ZymoBIOMICS Microbial Community Standard Material,  100  $\mu\text{L}$  EBV, and  100  $\mu\text{L}$  DENV-1 standard into a tube from step 4 from "Before Start" section. Add  250  $\mu\text{L}$  ASL buffer. 
- 4 Include a negative control for each batch of samples: a bead tube from step 4 from "Before Start" section with  500  $\mu\text{L}$  ASL buffer only.
- 5 Refill the dry ice compartment of the Bullet Blender if necessary. Load all samples into the Bullet Blender.
- 6 Set the speed at 12 and the time at 3. Press Start.
- 7 After beating, incubate samples at  95  $^{\circ}\text{C}$  for  00:15:00 .   

- 8 After incubation, beat the samples in the Bullet Blender (Refill the dry ice compartment if necessary) at speed 12 and time at 3.
- 9 Let the samples settle for  00:01:00 in the Bullet Blender and then repeat step 8. 
- 10 Centrifuge the bead tubes with lysate for  12000 x g, 4 $^{\circ}\text{C}$ , 00:05:00 . 



#### Note

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

## 2. INSTRUMENT SET UP

11

#### Note

KingFisher Flex only, if using KingFisher Duo Prime, go to 6. INSTRUMENT SET UP.

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

- 12 Ensure the program **IndiMag\_Pathogen\_KF\_Flex\_4wash** or the program has been downloaded and loaded onto the KingFisher Flex instrument.

## 3. SET UP THE PROCESSING PLATES

- 13 Set up the Tip Comb, Wash, and Elution 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          |
|  | Wash 3   | 4              | Deep-Well                                          | 80% ethanol         | 750 µL          |

|  | A      | B | C             | D                       | E      |
|--|--------|---|---------------|-------------------------|--------|
|  | 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 |

## 4. EXTRACTION

- 14 Add  20 µL of Proteinase K into wells of a deep-well plate (based on the number of samples). 
- 15 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.
- 16 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 (it can be mixed on a Hula mixer for  00:02:00 ). Add  695 µL mixture to each sample. Each well including sample lysate should be  985 µL . 2m  
- 17 Select the program **IndiMag\_Pathogen\_KF\_Flex\_4wash** on the instrument.
- 18 Start the run, then load the prepared plates into position when prompted by the instrument.

## 5. QUANTIFICATION AND STORAGE

- 19 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.
- 20 In a  6 mL microcentrifuge tube, use  1 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).

**NOTE:** If the DNA or RNA concentration measurement is too high, dilute  $2\ \mu\text{L}$  of sample in a new  $1.5\ \text{mL}$  tube with  $18\ \mu\text{L}$  nuclease-free water. Use  $3\ \mu\text{L}$  of the diluted sample for DNA or RNA quantification.

- 21 Proceed with sample testing following the REDI-NET SOP F-4 Fecal Testing or store at  $-20\ ^\circ\text{C}$  for less than 2 weeks.

#### Note

For long-term storage the sample needs to be stored at  $-80\ ^\circ\text{C}$  following the REDI-NET SOP F-3 Fecal Storage.

## 6. INSTRUMENT SET UP

22

#### Note

KingFisher Duo Prime only, if using KingFisher Flex, go to 2. INSTRUMENT SET UP.

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

- 23 Ensure the program IndiMag\_Pathogen\_KF\_Duo\_4wash has been downloaded and loaded onto the KingFisher Duo Prime instrument.

## 7. SET UP THE SAMPLE PLATE AND ELUTION STRIP

- 24 Set up the Sample Plate according to the table below:

|  | A      | B         | C       | D               |
|--|--------|-----------|---------|-----------------|
|  | Row ID | Plate Row | Reagent | Volume per well |

|  | A                 | B | C                       | D      |
|--|-------------------|---|-------------------------|--------|
|  | <b>Sample row</b> | A | Lysate and lysis buffer | 985 µL |
|  | <b>Wash 1</b>     | B | Buffer AW1              | 700 µL |
|  | <b>Wash 2</b>     | C | Buffer AW2              | 700 µL |
|  | <b>Wash 3</b>     | D | 80% ethanol             | 750 µL |
|  | <b>Wash 4</b>     | E | 100% ethanol            | 750 µL |
|  |                   | F | Tip comb                |        |
|  |                   | G | Empty                   |        |
|  |                   | H |                         |        |

25 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                      |
|--|-----------------|------------|---------------------|------------------------|
|  | <b>Strip ID</b> | <b>Row</b> | <b>Reagent</b>      | <b>Volume per well</b> |
|  | Elution         | A          | Nuclease-free water | 75 µL                  |

## 8. EXTRACTION

26 Add  20 µL of Proteinase K into wells of Row A (based on number of samples) of a new deep-well plate.



27 Transfer  270 µL supernatant without any particle carryover to the wells of the deep-well Row A containing proteinase K. This row becomes the Sample Row.



- 28 Add 135  $\mu\text{L}$  Buffer VXL, 540  $\mu\text{L}$  Buffer ACB, and 20  $\mu\text{L}$  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  $\mu\text{L}$  mixture to each sample. Each well including sample lysate should be 985  $\mu\text{L}$  .
- 29 Select the program **IndiMag\_Pathogen\_KF\_Duo\_4wash** on the instrument.
- 30 Start the run, then load the prepared plate and Elution Strip 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.

## 9. QUANTIFICATION AND STORAGE

- 31 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.
- 32 In a 0.6 mL microcentrifuge tube, use 1  $\mu\text{L}$  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.

**NOTE:** If the DNA or RNA concentration measurement is too high, dilute 2  $\mu\text{L}$  of sample in a new 1.5 mL tube with 18  $\mu\text{L}$  nuclease-free water. Use 3  $\mu\text{L}$  of the diluted sample for DNA or RNA quantification.

- 33 Proceed with sample testing following the REDI-NET SOP F-4 Fecal 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 F-3 Fecal Storage.

## APPENDIX 2. DNA and RNA Measurement using QUBIT FLUOROMETER 4.0

1m

### 34 DNA quantification:

According to the volume of sample used, add the 1x HS dsDNA Qubit Assay for a final volume of  200 µL (i.e., if using  1 µL of sample, add  199 µL of 1x HS dsDNA Qubit Assay). Vortex for 5 - 10 seconds, then incubate for  00:02:00 at  Room temperature before reading.

2m



### 35 RNA Quantification:

- 35.1 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               |

- 35.2 In a new  0.6 mL tube, mix  199 µL of Qubit HS RNA Assay working solution and  1 µL of the sample. Vortex for 5 - 10 seconds, then incubate for  00:02:00 at  Room temperature before reading.

2m





## Protocol references

### REFERENCES

#### REDI-NET Overview Summary

1. User Guide: Indical IndiMag Pathogen Kit user's manual
2. Indical Sample Pretreatment F2 (HB-2514-EN-001): For isolation of bacterial and viral DNA from Feces.
3. International Human Microbiome Standards Consortium, IHMS - quality protocol sop for fecal samples DNA extraction protocol Q, Code: IHMS\_SOP 06 V2.