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

**Created:** May 12, 2023

# B2- BLOOD PROCESSING

REDI-NET Consortium[1]

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## DISCLAIMER

This work is supported by the US Army Medical Research and Development Command under Contract No.W81XWH-21-C-0001, W81XWH-22-C-0093 and HT9425-23-C-0059. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army or Navy position, policy or decision unless so designated by other documentation.

## ABSTRACT

This protocol details blood processing.

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**PROTOCOL integer ID:** 81794

**Keywords:** BLOOD PROCESSING, WHOLE BLOOD AND BUFFY COAT LYSIS, CELL-FREE FLUID LYSIS (SERUM/PLASMA), FTA CARD LYSIS

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GUIDELINES

## OBJECTIVE

To outline procedures for total nucleic acid extraction from blood samples in various formats (whole blood, plasma, serum, buffy coat and dried blood spot on FTA cards).

## 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 blood samples to provide materials for downstream library preparation and sequencing for pathogen detection.

## 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.

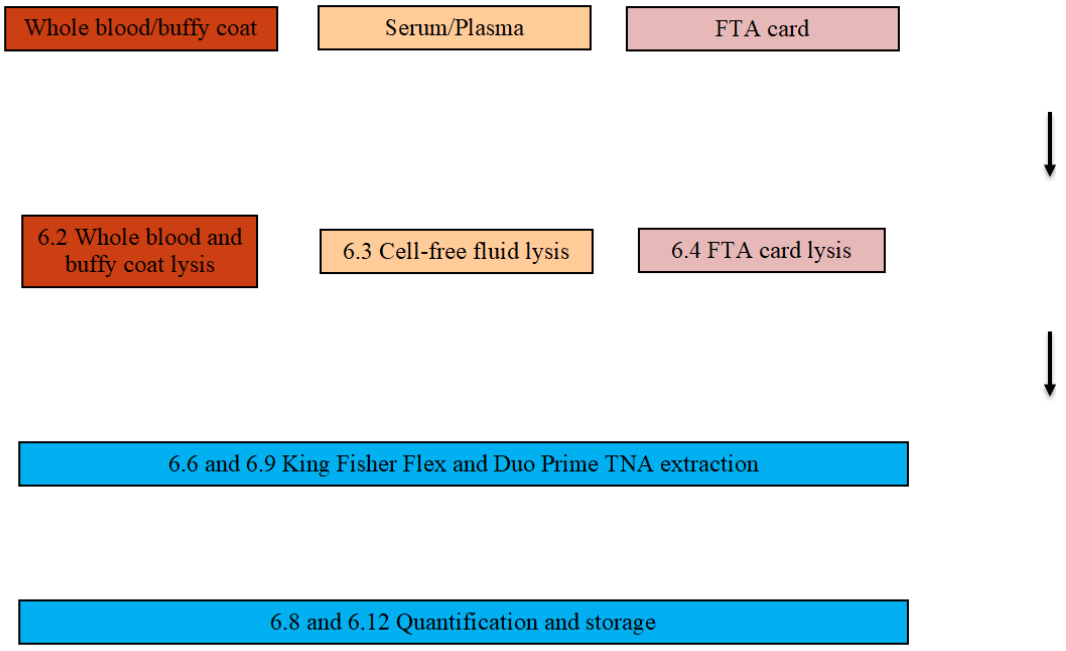
4. Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. When handling 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. WORKFLOW OF BLOOD SAMPLE PROCESSING




### APPENDIX 2. MEASURING SPOON FOR 0.1 MM BEATING BEADS

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

## APPENDIX 4. EXPECTED OUTCOMES

Expected result

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| | **Sample** | **Amount** | **Sample condition** | **Elution volume** | **DNA conc. (ng/ul)** | **RNA conc. (ng/ul)** |
| | Whole Blood | 200 uL | Frozen/Fresh | 75 | | |
| | Buffy coat | 200 uL | Frozen/Fresh | 75 | | |
| | Serum | 1.5 mL | Frozen/Fresh | 75 | | |
| | Plasma | 1.5 mL | Frozen/Fresh | 75 | | |
| | FTA card | Three 3 mm disc | Frozen/Fresh | 75 | | |

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 |
| | DynaMag-2 magnet | Invitrogen, 12321D or equivalent |
| | Hula sample mixer | ThermoFisher, 15920D |
| | 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 |

Equipment

| NAME | |
|---|---|
| **KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head** | |
| TYPE | Purification System |
| BRAND | Thermo Scientific™ |
| SKU | 5400630 |
| LINK | https://www.thermofisher.com/order/catalog/product/5400630 |

Equipment

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

Equipment

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

Equipment

| | |
|---|---|
| **DynaMag-2** | NAME |
| Magnet | TYPE |
| Invitrogen | BRAND |
| 12321D | SKU |
| https://www.thermofisher.com/order/catalog/product/12321D#/12321D | LINK |

Equipment

| | |
|---|---|
| **Hula mixer** | NAME |
| Mixer | TYPE |
| Invitrogen | BRAND |
| 15920D | SKU |
| Any rotator mixer | SPECIFICATIONS |

| | A | B | C |
|---|---|---|---|
| | **Material** | **Description** | **Mfg / Product #** |
| | IndiMag Pathogen Kit | w/o plastics, 384 reactions | Indical Bioscience, SP947257 |
| | Buffer ATL | 200 mL, Tissue Lysis Buffer | Qiagen, 19076 |
| | Reagent DX | 1 mL, Antifoaming Reagent | Qiagen, 19088 |
| | QIAcard FTA Wash Buffer | 25 mL, FTA card wash buffer | Qiagen, WB120112 |
| | UniCore Punch Kit 3.0 mm | 3.0 mm, FTA card puncher and mat | Qiagen, WB100039 |
| | 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 |

| | A | B | C |
|---|---|---|---|
| | RNase-Free Microfuge Tubes | Nonstick, 2.0 mL | ThermoFisher, AM12475 |
| | 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 |
| | Zika virus (ZIKV) positive control | For TNA extraction positive control | NMRC made |
| | Human gammaherpesvirus (EBV) positive control | For TNA extraction positive control | NMRC made |
| | Forceps | 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 |
| | Dry ice | To maintain cold chain during sample handling | Locally sourced |
| | Ice bucket | To contain the dry ice and regular 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 |

**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.*

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

Buffer AL, Lysis buffer **Qiagen Catalog #19076**

Reagent DX **Qiagen Catalog #19088**

QIAcard FTA Wash Buffer **Qiagen Catalog #WB120112**

UniCore Punch Kit 3.0 mm **Qiagen Catalog # WB100039**

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™ Flex™ Systems Consumables, KingFisher 96 KF microplate (200µL) **Thermo Fisher 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 **Applied Biosystems (ThermoFisher 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**

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 Assay Kit **Thermo Fisher Scientific Catalog #Q33230**

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

Qubit assay tubes **Thermo Fisher Scientific Catalog #Q32856**

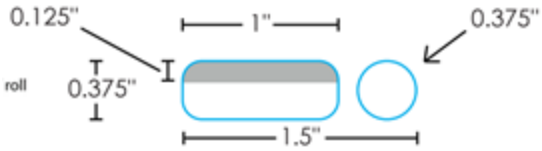
RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific Catalog #AM9780**

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

## APPENDIX 5. 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 |
| | Cryo-labels | 667 1.00" x 0.38" Cap & Wrap CryoLabel® w/0.375" Cap, Blanks, 1" Core<br><br>Color bar breakdown:<br>Grey - 31,24,25,0 | Electronic Imaging Materials, #335774-COLOR |

| | A | B | C |
|---|---|---|---|
| | | Orange - 0,80,95,0<br>Blue - 85,50,0,0<br>Brown - 35,60,80,25<br>Yellow - 0,0,100,0 | |
| | 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

## 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 2) to Thermo Scientific 1.5 ml Screw Cap Micro Tubes.
4. 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).
5. 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.
6. MagAttract Suspension G from IndiMag pathogen kit needs to be vortexed thoroughly for 00:03:00 (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.
7. Aliquot nuclease-free water in big bottle into a few 15 ml tubes for preparing TNA elution in KingFisher Flex or KingFisher Duo Prime to avoid cross-contamination.

Note

***NOTE:*** *Check* ***APPENDIX 1 THE WORKFLOW OF BLOOD SAMPLE PROCESSING*** *for the overview of processing blood samples in different formats when using this SOP for the first time. The lysis for different formats of blood samples is described in three sections in this SOP: section 6.2 for processing whole blood and buffy coat; section 6.3 for processing serum and plasma; section 6.4 for processing FTA card.*

## 1. WHOLE BLOOD AND BUFFY COAT LYSIS

1

Note

***NOTE:*** *Blood samples treated with EDTA, citrate, or heparin as an anticoagulant can be used for nucleic acid purification. Samples can be either fresh or frozen, provided they have not been freeze-thawed more than once. Freeze-thawing more than once can lead to denaturation and precipitation of proteins, resulting in a potential reduction in viral titers, and therefore, reduced yields of viral nucleic acids.*

2 Add 200 µL sterile 1x PBS to a 200 µL whole blood or buffy coat sample.

Note

If provided whole blood or buffy coat has a total volume less than 200 µL, add sterile 1x PBS to make up the 200 µL.

3 Transfer 400 µL blood and 1x PBS mixture to the bead tubes prepared in step 3 of Before Start section under the Guidelines & Warnings tab. Add 100 µL of ATL-DX buffer.

4 Include a positive control for each batch of samples: transfer 37.5 µL ZymoBIOMICS Microbial Community Standard Material, 100 µL EBV, and 100 µL ZIKV standard into a tube from step 3 of Before Start section. Add 100 µL ATL-DX buffer and 162.5 µL sterile 1x PBS.

5 Include a negative control for each batch of samples: a bead tube from step 3 of Before Start section with 100 µL ATL-DX buffer and 400 µL sterile 1x PBS.

6 Load all samples into the Bullet Blender (refill dry ice if necessary).

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

8 Let the samples settle for 00:01:00 in the Bullet Blender and then repeat step 7. 1m

Note

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

## 2. CELL-FREE FLUID LYSIS (SERUM/PLASMA)

9 Add up to 1.5 mL fluid sample to a 2 ml microcentrifuge tube (depending on available volume, no less than 0.5 mL) and centrifuge the tube for 00:05:00 at maximum speed (12000- 14000 xg). 5m

10 Use a pipet to remove the supernatant.

11 Add 500 µL ATL-DX buffer and resuspend the pellet in by vortexing.

12 Transfer the entire pellet resuspension to the bead tubes prepared in step 3 of Before Start section.

13 Include a positive control for each batch of samples: transfer 37.5 µL ZymoBIOMICS Microbial Community Standard Material, 100 µL EBV, and 100 µL ZIKV standard into a tube from step 3 of Before Start section. Add 250 µL ATL-DX buffer.

14 Include a negative control for each batch of samples: a bead tube from step 3 of Before Start section with 500 µL ATL-DX buffer.

15 Load all samples into the Bullet Blender (refill dry ice if necessary).

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

17 Let the samples settle for 00:01:00 in the Bullet Blender and then repeat step 16. 1m

Note

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

## 3. FTA CARD LYSIS

18 Punch FTA card with dried blood spots with a 3.0mm single-hole puncher.

19 Use a forceps to place three card punches of the same sample into a bead tube prepared in step 3 of Before Start section.

20 Add 20 µL Proteinase K and 200 µL QIAcard FTA Wash Buffer and 200 µL ATL-DX buffer to 15s the sample. Mix immediately by vortexing for 00:00:15.

21 Include a positive control for each batch of samples: transfer 37.5 µL ZymoBIOMICS Microbial Community Standard Material, 100 µL EBV, and 100 µL ZIKV standard into a tube from step 3 of Before Start section. Add 125 µL QIAcard FTA Wash Buffer and 125 µL ATL-DX buffer and 20 µL proteinase K.

22 Include a negative control for each batch of samples: a bead tube from step 3 of Before Start section with 200 µL QIAcard FTA Wash Buffer and 200 µL ATL-DX buffer and 20 µL proteinase K.

23 Incubate in a thermomixer at 1400 rpm, 56°C, 01:00:00.

24 Refill the dry ice compartment of the Bullet Blender if necessary. After incubation, load all samples into the Bullet Blender.

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

26 Let the samples settle for 1 minute in the Bullet Blender and then repeat step 25.

Note

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

## 4. INSTRUMENT SET UP

27

Note

NOTE: KingFisher Flex only, if using KingFisher Duo Prime, go section 8

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

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

## 5. SET UP THE PROCESSING PLATES

29 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 |
| | **Wash 2** | 3 | Deep-Well | Buffer AW2 | 700 µL |
| | **Wash 1** | 2 | Deep-Well | Buffer AW1 | 700 µL |

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | **Sample** | 1 | Sample Lysate | Lysate and lysis buffer | 985 µL |

## 6. EXTRACTION

30 Centrifuge the bead tubes with lysate from the sample lysis step for 12000 x g, 00:05:00. 5m

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

Note

***NOTE**: Skip this step for the samples from the FTA card of section 3.*

32 Transfer 270 µL supernatant (for FTA card samples of section 3, transfer 290 µL supernatant) without any particle carryover to the wells of the Deep-well plate. This plate becomes the Sample Plate.

33 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

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

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

## 7. QUANTIFICATION AND STORAGE

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

37 In 0.6 mL microcentrifuge tubes, use 1 µL total nucleic acid from whole blood/buffy coat samples, or 3 µL total nucleic acid from FTA card/cell-free fluid samples, for DNA and RNA concentration measurements using Qubit 4 Fluorometer following manufacturer instructions.

Note

**Kits needed**: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see Appendix 3 and Appendix 4).

**NOTE**: If the concentration measurement of the TNA is too high, dilute 2 uL of the sample in a new 1.5 mL tube with 18 µL nuclease-free water. Use 3 µL of the diluted sample for DNA or RNA quantification.

38 Proceed with sample testing following the REDI-NET SOP B-4 Blood 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 B-3 Blood Storage.

## 8. INSTRUMENT SET UP

39

Note

NOTE: KingFisher Duo Prime only, if using KingFisher Flex, go to section 4

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

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

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

41 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 | | |

42 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 |
|---|---|---|---|---|
| | **Strip ID** | **Row** | **Reagent** | **Volume per well** |
| | **Elution** | A | Nuclease-free water | 75 µL |

## 10. EXTRACTION

43 Centrifuge the bead tubes with lysate from the sample lysis step for 12000 x g, 00:05:00. 5m

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

Note

***NOTE**: Skip this step for the samples from the FTA card of section 3.*

45 Transfer 270 µL supernatant (for FTA card samples of section 3, transfer 290 µL supernatant) without any particle carryover to the wells of the Deep-well Row A. This row becomes the Sample Row.

46 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 and avoid foaming. Add 695 µL mixture to each sample. Each well including sample lysate should be 985 uL.

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

48 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.

## 11. QUANTIFICATION AND STORAGE

49 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

50 In 0.6 mL microcentrifuge tubes, use 1 µL total nucleic acid from whole blood/buffy coat samples, or 3 µL total nucleic acid from FTA card/cell-free fluid samples, for DNA and RNA concentration measurements using Qubit 4 Fluorometer following manufacturer instructions.

Note

**Kits needed**: Qubit 1X dsDNA HS Assay Kit and Qubit RNA HS Assay Kit. (see **Appendix 3** and **Appendix 4** in Guidelines & Warnings tab)
***NOTE:*** *If the concentration measurement of the TNA is too high, dilute 2 uL of the sample in a new 1.5 mL tube with 18 uL nuclease-free water. Use 3 uL of the diluted sample for DNA or RNA quantification.*

51 Proceed with sample testing following the REDI-NET SOP B-4 Blood 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 B-3 Blood Storage.

## APPENDIX 3. DNA and RNA Measurement using QUBIT FLUOROMETER 4.0

4m

52 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 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.

53 RNA Quantification:

53.1 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 |

53.2 In a new 0.6 ml tube, mix 197 µL of Qubit HS RNA Assay working solution and 3 µL o 2m the sample. Incubate for 00:02:00 at Room temperature before reading.