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Version 1

## B2- BLOOD PROCESSING V.1

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**Protocol status:** Working

**We use this protocol and it's working**

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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, protocol details blood processing, protocol detail, processing, protocol

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## Disclaimer

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## Abstract

This protocol details blood processing.

## 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

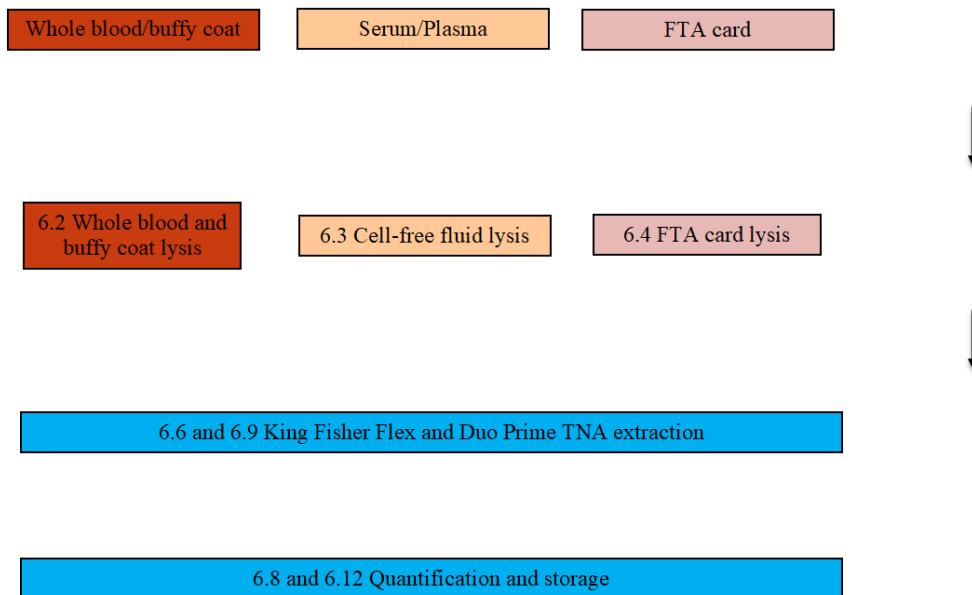
1. Proteinase K is stable for at least 1 year after delivery when stored at  Room temperature (15-25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, storage at  2-8 °C is recommended. Do not add Proteinase K directly to the Buffer VXL mixture! This can cause clogs or precipitates.
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  $\mu$ L .



## **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

<b>KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head</b>		NAME
Purification System		TYPE
Thermo Scientific™		BRAND
5400630		SKU
<a href="https://www.thermofisher.com/order/catalog/product/5400630">https://www.thermofisher.com/order/catalog/product/5400630</a>		LINK

## Equipment

<b>Bullet Blender 24 Gold (1.5 mL snap and screw cap tubes, 4°C cooling)</b>		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

<https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238><sup>LINK</sup>

## Equipment

**DynaMag-2**

NAME

Magnet

TYPE

Invitrogen

BRAND

12321D

SKU

<https://www.thermofisher.com/order/catalog/product/12321D#/12321D><sup>LINK</sup>

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 <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



	A	B	C
	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
	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

	A	B	C
	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**

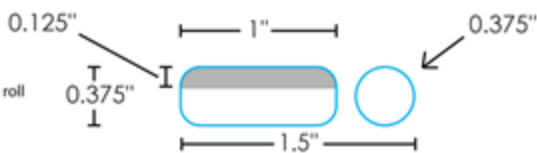
KingFisher® Duo and KingFisher® Duo Prime Consumables, 12-tip comb, for Microtiter 96 Deepwell plate **Thermo Fisher Catalog #97003500**

- 
 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

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

Troubleshooting

Safety warnings

**! RISK AND PERSONAL PROTECTION**

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








## Before start

### **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  $\mu\text{L}$  sterile 1x PBS to a  200  $\mu\text{L}$  whole blood or buffy coat sample.

### Note

If provided whole blood or buffy coat has a total volume less than  200  $\mu\text{L}$ , add sterile 1x PBS to make up the  200  $\mu\text{L}$ .

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


4 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  $\mu\text{L}$  ZIKV standard into a tube from step 3 of Before Start section. Add  100  $\mu\text{L}$  ATL-DX buffer and  162.5  $\mu\text{L}$  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  $\mu\text{L}$  ATL-DX buffer and  400  $\mu\text{L}$  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).
- 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).

5m





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 the sample. Mix immediately by vortexing for 00:00:15 .

15s



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
	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

2m






mixed on a Hula mixer for  00:02:00 ). Add  695  $\mu\text{L}$  mixture to each sample. Each well including sample lysate should be  985  $\mu\text{L}$  .



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 instrument and cover the plate or transfer the eluate to the final tube or plate of choice for final storage.


35m

37 In 0.6 mL microcentrifuge tubes, use  1  $\mu\text{L}$  total nucleic acid from whole blood/buffy coat samples, or  3  $\mu\text{L}$  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  $\mu\text{L}$  of the 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.

38 Proceed with sample testing following the REDI-NET SOP B-4 Blood 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 °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 µL.






- 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  $\mu\text{L}$  total nucleic acid from whole blood/buffy coat samples, or  3  $\mu\text{L}$  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  $\mu\text{L}$  of the 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.

- 51 Proceed with sample testing following the REDI-NET SOP B-4 Blood 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 °C following the REDNET 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 of the sample. 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 B1 (HB-2533-EN-001): For Difficult-to-Lyse Bacteria in Whole-Blood or Pretreated Tissue.
3. Indical Sample Pretreatment B2 (HB-2534-EN-001): For Difficult-to-Lyse Bacteria in Cell-Free Fluids.