DNA extraction from 5uL mouse blood samples (KingFisher Flex 96-well)

Petra Schneider[1], Charlotte Repton[1], Sarah Reece[1]

[1]University of Edinburgh

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1 Works for me

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Petra Schneider

ABSTRACT

DNA extraction from 5uL mouse blood samples, for subsequent qPCR to detect *Plasmodium* parasites within the blood. Using KingFisher Flex (96 well) system with MagMAX™-96 DNA Multi-Sample Kit. Protocol to import into BindIt Software attached.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Schneider, P. et al. (2018) 'Adaptive plasticity in the gametocyte conversion rate of malaria parasites', PLoS Pathogens, 14(11). doi: 10.1371/journal.ppat.1007371.

GUIDELINES

Please note that DNA was extracted using the semi-automatic Kingfisher Flex Magnetic Particle Processor and the MagMax 96-DNA multisample kit for DNA (4413021/4413022Thermo Fisher Scientific) but with adjustments to the manufacturer's protocols (standard protocols 4413021DWblood) to improve recovery.

MATERIALS

NAME	CATALOG #	VENDOR
MagMAX™-96 DNA Multi-Sample Kit	4413021	Thermo Fisher
KingFisher™ Flex™ Systems Consumables, KingFisher Flex Microtiter Deepwell 96 plate, sterile	95040460	Thermo Fisher
KingFisher™ Flex™ Systems Consumables, KingFisher 96 tip comb for DW magnets	97002534	Thermo Fisher
KingFisher™ Flex™ Systems Consumables, KingFisher 96 KF microplate (200µL)	97002540	Thermo Fisher

MATERIALS TEXT

In addition you will need: isopropanol, absolute ethanol, citrate saline (0.85 % w/v NaCl, 1.5% w/v trisodium citrate dihydrate) and general lab materials (plastics, pipettes, pipette tips)

Sample Collection

1 **Dispense 150 µL of citrate saline** (0.85 % w/v NaCl, 1.5% w/v trisodium citrate dihydrate) into 0.2 mL strip tubes or 96-well plates, one well per sample. Cover tubes with lids, or plate with a plastic film (*the thick version, not the optical film for qPCRs – that one will NOT come off*).

2 **Take a 5 µL blood sample** (we use glass capillaries) and dispense the blood immediately into the citrate saline. Repeat for all samples.

3 **Mix** with pipette and replace lid; or replace lid and mix by carefully tapping the tube.

4 **Spin down and remove supernatant.** Centrifuge 3 minutes at 4ºC. Remove supernatant, using clean pipette tip for each sample.

5 **Freeze samples** at -20C (short term), -70°C (long-term) or immediately extract DNA.

Prepare for DNA extraction

6 **Defrost your samples.**

7 **Get required plastics.** You will need 4 Kingfisher DeepWell (DW; Cat# 95040460) plates, 2 Kingfisher 96 (KF; Cat# 97002540) plates and 1 DW tip comb (Cat# 97002534).

8 **Prepare reagents in kit only if opening a new MagMax 96-DNA multisample kit** (Cat# 4413021), Add the appropriate amount of ethanol or isopropanol to the wash buffers (see table below) and mark the bottles clearly by crossing out the word “concentrate”, ticking the small checkbox and writing your name/initials and the date.

Reagent	Location	Action
Lysis buffer	Bench	None
Wash 1	Bench	Add 5.4 mL 100% isopropanol
Wash 2	Bench	Add 40 mL 100% ethanol
Elution Buffer 1	Bench	None
Elution Buffer 2	Bench	None
DNA Binding beads	4 °C fridge	None
Proteinase K solution	-20 °C freezer	None
PK buffer	Bench	None

New MagMax 96-DNA multisample kit - Preparation of reagents (Thermofisher 4413021)

Note: some of the bench-top reagents crystallise when the ambient temperature is low (below ~18°C). To combat this, set a heat block to 25°C before beginning and place the room temperature reagents on it until ready to use, remembering to mix well!

9 **Prepare Proteinase K (ProK) mix.** In a sterile, DNase/RNase-free eppendorf tube or vial mix **8 mL** of ProK solution and **42uL** of PK buffer (total = 50 mL) per well (i.e. sample), plus 10% extra for errors.

10 **Prepare bead mix.** In a sterile, DNase/RNase-free eppendorf tube, mix **16 mL** of DNA binding beads and **4 mL** of nuclease free water (total = 20 mL) per well (i.e. sample), plus 10% extra for errors.

11 **Fill the plates** as shown in the table:

Plate (type)	Contents /well
Tip plate(KF)+DWTip Comb	N/A
Sample (DW)	50 uL ProK mix (prepared in step 6) 5 uL sample
Wash 1 (DW)	150 uL wash 1 solution
Wash 2_1 (DW)	150 uL wash 2 solution
Wash 2_2 (DW)	150 uL wash 2 solution
Elution (KF)	75 uL elution buffer 1

Plate filling instructions

Note: Use protK mix to dilute 5uL blood sample before transfer – easier pipetting.

DNA extraction

12 **Connect the Kingfisher instrument** to the computer using USB cable (*note: this should already be there)*.

13 **Open the BindIt software**. Select 'Your Kingfisher Machine' from the drop-down 'connect' list to connect to your Kingfisher instrument (*note: connection has likely established automatically)*.

14 **Select the protocol** (see step 22), and click on the 'start' (green arrow) button on *the BindIt software*.

15 **Load the plates into the machine.** The machine will turn the turntable and prompt you to load the plates in the correct order. Press the 'start' button on *the instrument* to confirm you have loaded the current plate, this tells the machine to progress to the next plate. Make sure to line up the A1 well of the plate with the marked corner on the turntable position.

16 **Follow dispense step instructions.** The run will begin once all plates are loaded. The instrument will prompt you to add the correct reagents at the dispense steps (grey cells in machine protocol, below). The first dispense step is roughly 20min after start-time. A guide to the approximate length of time until a step ends (so that you can work out how long between dispense steps) is also included in the machine protocol table below.

17 **Remove plates** when the run has ended (as prompted by machine). Export AND SAVE the run details as a PDF (automatically opened in the software) – it contains details of the times each steps were begun as well as temperatures at frequent intervals, *if something has gone wrong this may give you the answer why. Do not yet discard.*

18 **Check, seal and label DNA extracts.** Check there is liquid in the elution plate (*if not, something has gone wrong!)*. Seal the elution plate with a plastic film (make sure it is freezer-suitable and do not use the optical qPCR films – these do NOT come off) and label it carefully.

19 **Freeze** at -20°C or use immediately for qPCR. For use in qPCRs, we recommend diluting at least 1 volume extracted DNA + 2 volumes water to remove the effects of inhibitors.

20 **Discard all plates, except the elution plate**. If you haven't used every well, you can keep them to be reused (be sure to use different, clean wells to the ones you have already used). And, if you do keep them for a while – remove liquids from used wells before storage.

Kingfisher Protocol

21 **Optimized machine Protocol**

File to import into your BindIt Software: DNA5uLMouseBlood.bdz

Summary:

Plates (add to BindIt software under the 'Layout' tab):

- Tip plate (KF 96 plate with DW 96 tip comb inserted)
- Sample plate (DW microtiter plate)
- Wash 1 (DW microtiter plate)
- Wash 2_1 (DW microtiter plate)
- Wash 2_2 (DW microtiter plate)
- Elution (KF 96 plate)

Protocol (add to BindIt software under the 'Protocol' tab):

Step name	Description	Plate	Step end (approx. time from start)
Pick up tip		Tip plate	start
Lysis 1	30s bottom mix Temperature 20°C	Sample	30s
Lysis 2	Heating during mixing 62°C, 10 min paused, tip above well/tube surface, postmix 30s slow.	Sample	11 min
Lysis 3	Heating during mixing 62°C, 10 min paused, tip above well/tube surface.	Sample	21 min
Add lysis buffer	Dispense. Message: "Add 200 mL lysis buffer."	Sample	(~ 3 min) 24 min
Lysis 4	3 min 30s bottom mix. Temperature 20°C	Sample	27 min 30s
Add beads	Dispense. Message: "Add 20 mL beads."	Sample	(~ 5 min) 32 min 30s
Mix beads	3 min bottom mix. Temperature 20°C	Sample	35 min 30s
Add isopropanol	Dispense. Message: "Add 240 mL ispropanol."	Sample	(~ 3 min) 38 min 30s
Mix isopropanol	5 min half mix; collect beads count 5 time 30s. Temperature 20°C	Sample	46 min
Wash 1	Release beads 30s bottom mix; [mix 10s half mix, mix 10s fast] x 3 loops; collect beads count 3, time 1s. Temperature 20°C	Wash 1	47 min 33s
Wash 2_1	Release beads 30s bottom mix; [mix 10s half mix, 10s fast] x 3 loops; collect beads count 3, time 1s. Temperature 20°C	Wash 2_1	49 min 6s
Wash 2_2	Release beads 30s bottom mix; [mix 10s half mix, 10s fast] x 3 loops; collect beads count 3, time 1s. Temperature 20°C	Wash 2_2	50 min 39s
Dry	Dry time 3 min, outside well/tube.	Wash 2_2	53 min 39s
Elution 1	Release beads 30s bottom mix; heating during mixing 72°C, preheat, mix 5 min slow; postmix 4 min fast.	Elution	63 min 9s
Add elution buffer 2	Dispense. Message: "Add 75 mL elution buffer 2."	Elution	(~ 3 min) 66 min 9s
Elution 2	30s bottom mix, 2 min medium; collect beads count 5, time 30s. Temperature 20°C	Elution	71 min 9s
Leave tip in wash 2_2.		Wash 2_2	

KingFisher Protocol for DNA extraction from 5uL mouse blood samples (BindIt software) with added time frame for extraction run.

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