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Protocol: Purification of DNA from Whole Blood using the QIAamp Blood Midi Kit (Spin Protocol) + QC with Qubit fluorometer V.1

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We use this protocol and it's working

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Disclaimer

Abstract

The QIAamp DNA Blood Midi procedure yield pure DNA ready for direct restriction digestion or amplification. With a suitable centrifuge rotor, **eight samples** can be prepared simultaneously in about **1.5 hours** with approximately **30 minutes of hands-on time.**

QIAamp DNA Blood Midi Kits are suitable for use with whole blood that has been treated with EDTA, citrate, or heparin, and samples may be fresh or frozen. Separation of leukocytes is not necessary. The procedure require no phenol/chloroform extraction or alcohol precipitation and involve minimal handling.

DNA is eluted in Buffer AE or water and is ready for direct addition to PCR or other enzymatic reactions, or it **can be safely stored at –30 to –15°C** for later use.

The purified DNA is virtually free of protein, nucleases, and other contaminants or inhibitors of downstream applications.

DNA **ranges in size up to 50 kb**, with fragments of **approximately 30 kb predominating**.

	QIAamp DNA Blood Kits			
Leukocytes	Midi		Maxi	
per ml	µg	%	µg	%
2.5×10^5	3.0	90.9	15.8	95.8
1.0×10^6	11.8	89.4	60.4	91.5
5.0×10^6	62.4	94.5	312.0	94.5
1.0×10^7	116.3	88.1	624.6	94.6

Genomic DNA was purified from 2 ml (Midi) and 10 ml (Maxi) of whole blood and eluted in 300 µl and 1 ml, respectively, of Buffer AE. Percentage figures are calculated on the basis of theoretical yields.

Source: QIAamp DNA Blood Midi/Maxi Handbook

Attachments

PDF

HB-0339-003-1090244-...

463KB

Materials

Equipment and Reagents to Be Supplied by User

- Suitable lab coat, disposable gloves
- Pipets + tips for each: P5000, P1000, P200, P10
- Centrifuge capable of attaining 4500 x g (5000 rpm), equipped with a swing-out rotor and buckets that can accommodate 15 ml centrifuge tubes
- 15 ml Falcon tubes
- Racks
- Water bath, heated to 70°C
- Vortex
- Mini centrifuge
- Stiled water
- Ethanol (96–100%): Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone
- Paper towels for cleaning
- Spray bottles with ethanol for cleaning
- Biohazard waste bags
- Airflow Benchtop Hood
- Fridge
- Freezer
- AC for the lab
- Access to Ice
- Buckets for Ice
- Qubit™ Fluorometer (4 or flex)
- Qubit™ 1X dsDNA High Broad Range (BR) Assay Kits: Working solution + Assay standards
- Qubit Assay Tubes

Instruments and accessories	Quantity	Cat. No.
Qubit 4 Fluorometer		
Qubit 4 Fluorometer (w/ Wi-Fi)	1 instrument	Q33238
Qubit 4 Quantitation Starter Kit (w/ Wi-Fi)	1 kit	Q33239
Qubit 4 NGS Starter Kit (w/ Wi-Fi)	1 kit	Q33240
Qubit 4 RNA IQ Starter Kit (w/ Wi-Fi)	1 kit	Q33241
Qubit 4 Protein BR Assay Starter Kit (w/ Wi-Fi)	1 kit	A51292
Qubit Assay Tubes	500 tubes	Q32856
Qubit 4 System Verification Assay Kit	50 assays	Q33237
Qubit Flex Fluorometer		
Qubit Flex Fluorometer	1 instrument	Q33327
Qubit Flex NGS Starter Kit	1 kit	Q45893
Qubit Flex Quantitation Starter Kit	1 kit	Q45894
Qubit Flex Assay Tube Strips	125 tube strips	Q33252
Qubit Flex Assay Reservoirs	100 reservoirs	Q33253
Qubit Flex System Verification Assay Kit	25 assays	Q33254
Qubit Flex Fluorometer with SAE Software for 21 CFR Part 11 Support	1 package	Q45895
Qubit Flex SAE Software for 21 CFR Part 11 Support	1 license	Q31994

Qubit 4 (1 sample) and Qubit flex (8 samples)

Kit Contents

QIAamp Midi Spin Columns
Collection Tubes (15 ml)
Buffer AL*
Buffer AW1* (concentrate)
Buffer AW2 (concentrate)
Buffer AE
QIAGEN® Protease: Resuspension volume 4.4 ml or 5.5 ml depending on the kit units (20 or 100)

QIAamp DNA Blood Midi Kit	(20)	(100)
Catalog no.	51183	51185
Number of preps	20	100
QIAamp Midi Spin Columns	20	100
Collection Tubes (15 ml)	20	100
Buffer AL*	2 x 33 ml	330 ml
Buffer AW1* (concentrate)	19 ml	98 ml
Buffer AW2 (concentrate)	17 ml	81 ml
Buffer AE	15 ml	60 ml
QIAGEN® Protease	1 vial†	4 vials‡
Selection Guide	1	1

* Not compatible with disinfecting agents containing bleach. Contains a chaotropic salt. Take appropriate safety measures, and see page 5 for further safety information.
† Resuspension volume 4.4 ml
‡ Resuspension volume 5.5 ml

Preparation of reagents

- QIAGEN **Protease** (store at 2–8°C stable for 2 months, storage –30 to –15°C will prolong the life)
  Pipet 4.4 ml distilled water into the vial of lyophilized QIAGEN Protease provided in the QIAamp DNA Blood Midi Kit (20), or 5.5 ml in the QIAamp DNA Blood Midi Kit (100), as indicated on the labels.

- **Buffer AL*** (store at room temperature 15–25°C, estable 1 year)

Mix Buffer AL thoroughly by shaking before use.

- **Buffer AW1** (store at room temperature, 15–25°C, estable 1 year)

Supplied as a concentrate. Add the appropriate amount of ethanol (96–100%) as indicated on the bottle **(only first time)**

- **Buffer AW2** (store at room temperature, 15–25°C, estable 1 year)

Supplied as a concentrate. Add the appropriate amount of ethanol (96–100%) as indicated on the bottle **(only first time)**

Troubleshooting

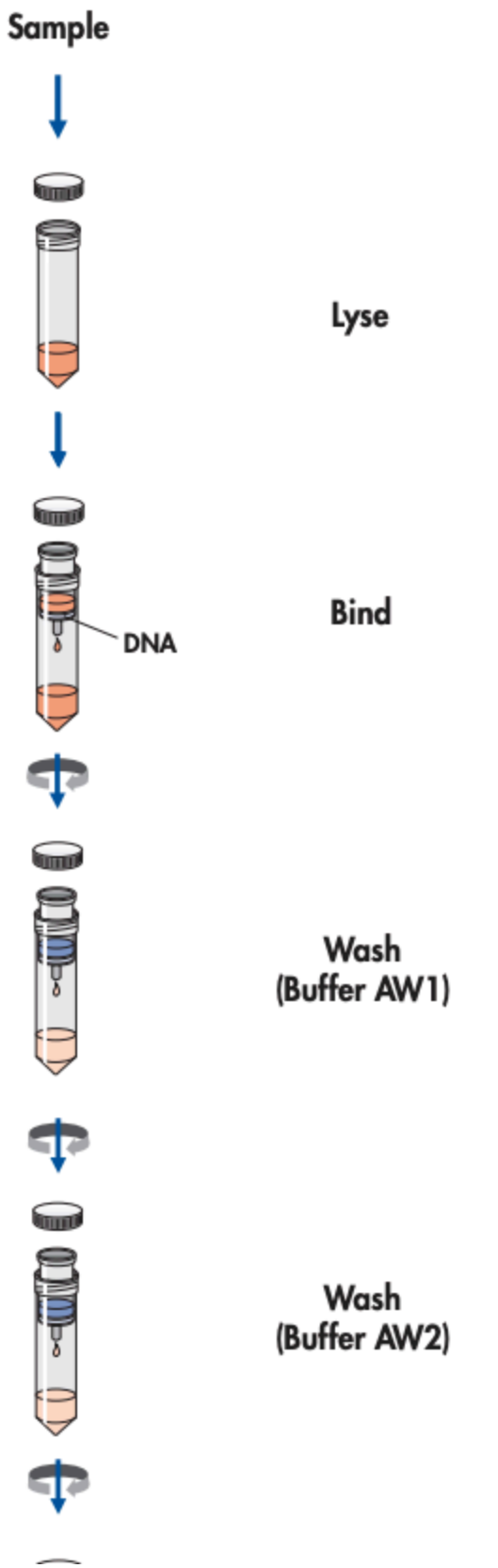
Before start

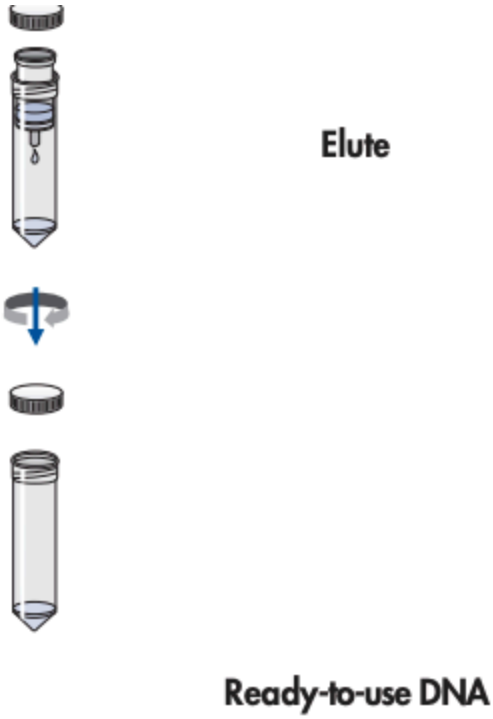
■ Equilibrate samples to r**oom temperature (15–25°C)** before starting.
■ Prepare a 70°C water bath (**critical initial step** and must precede all others in the protocol, Failure to execute prior to proceeding may compromise the **integrity and validity** of the entire procedure).
■ **Ensure** that Buffer AW1, Buffer AW2, and QIAGEN Protease have been **prepared** (Detailed on "materials").
■ If a precipitate has formed in Buffer AL, redissolve by incubating at 56°C.

DNA extraction procedure

1

Spin procedure





Summarized procedure

2 Pipet 200 µL **QIAGEN Protease** into the bottom of a 15 ml centrifuge tube. Add 2 mL Sample and mix briefly.

3 Add 2.4 mL **Buffer AL**, and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min (Check that the tubes are tightly closed).

4 **Incubate** at 70 °C **10 min.**
(During this time, store Protease in the fridge)

5 Carefully transfer (just squirt solution in middle of the membrane, don't need to go inside the tube with the tip) **one half** (aprox 2-3 mL) of the solution onto the **QIAamp Midi column** placed in a 15 ml centrifuge tube, cerefully **not to moistening the rim**.

5.1 With the cap tightly closed, **centrifuge** 3000 rpm (1850 x g) for 3min.

6 Add 2 mL **ethanol** (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.

6.1 Remove the QIAamp Midi column, **discard the filtrate** (dab the rim to remove any exccess of liquid), and place the QIAamp Midi column back into the 15 ml centrifuge tube. Load the remainder of the solution onto the QIAamp Midi column.

6.2 With the cap tightly closed, **centrifuge** 3000 rpm (1850 x g) for 3min.

7 Remove the QIAamp Midi column, **discard the filtrate**, and place the QIAamp Midi column back into the 15 ml centrifuge tube.

7.1 Add 2 mL **Buffer AW1** (carefully, without moistening the rim) to the QIAamp Midi column.

7.2 With the cap tightly closed, **centrifuge** at 5000 rpm (4500 x g) for 1 min.

8 Add 2 mL **Buffer AW2** (carefully, without moistening the rim) to the QIAamp Midi column.

8.1 With the cap tightly closed, **centrifuge** at 5000 rpm (4500 x g) for 15 min.

9 Place the QIAamp Midi column in a **clean 15 ml centrifuge tube**, and **discard** the collection tube containing the filtrate.

9.1 Pipet 300 µL **Buffer AE** directly onto the membrane of the **QIAamp Midi column** and close the cap.

10 **Centrifuge** at 5000 rpm (4500 x g) for 2 min.

11 Pipet 300 µL **Buffer AE** directly onto the membrane of the **QIAamp Midi column** and close the cap.

11.1 **Incubate** at room temperature for **5 min**.

12 **Centrifuge** at 5000 rpm (4500 x g) for 2 min.

Sample QC procedure (Qubit Fluorometer)

13 **STANDARDIZE THE QUBIT** (every time you use it or once a week according to lab protocol)

13.1 Get 0.2 mL Qubit tubes.

13.2 In one strip, add 10 mL of BR Standard 1.

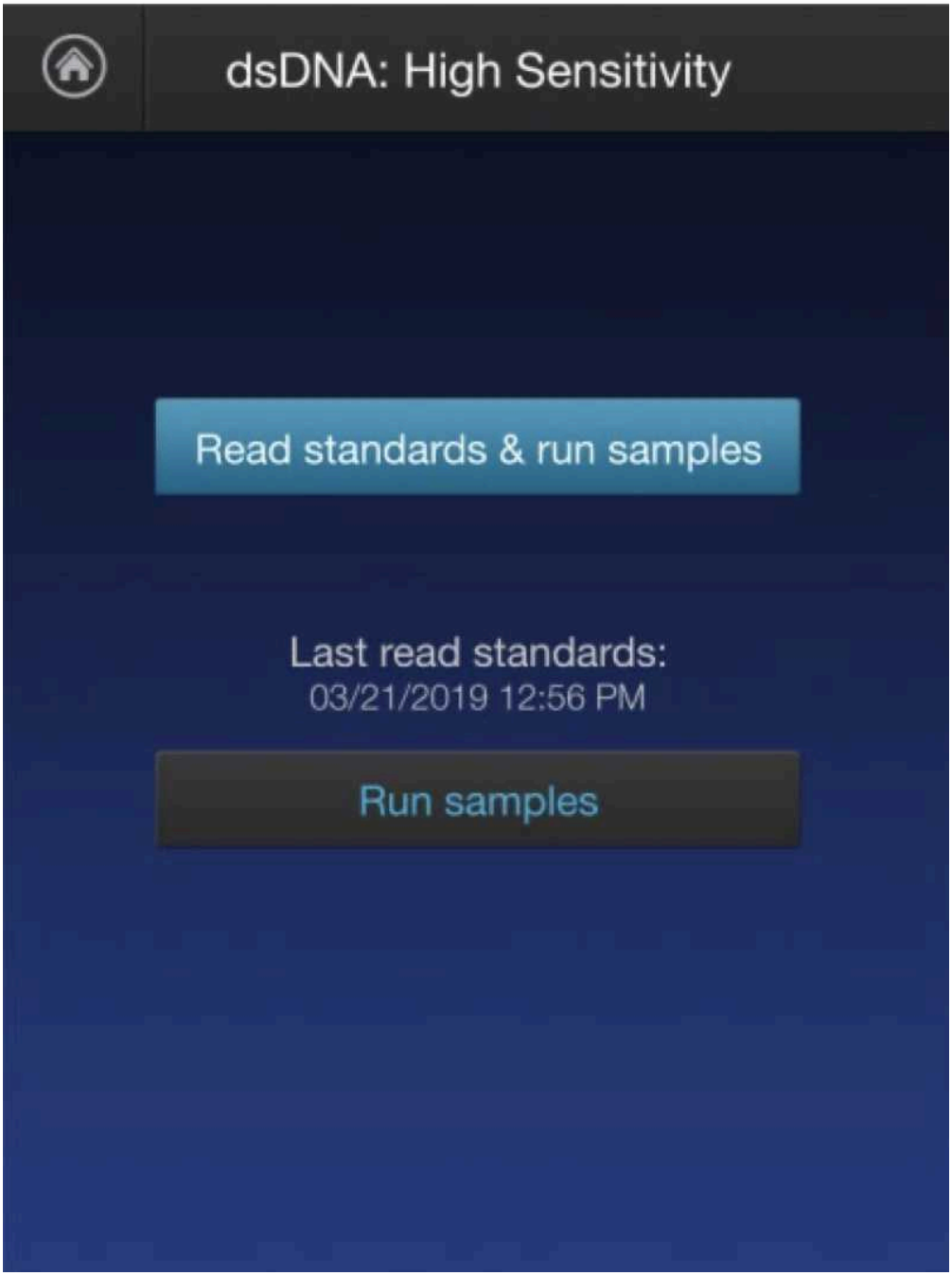
13.3 In the other strip, add 10 mL of BR Standard 2.

13.4 Add 190 µL of the working solution to all of the tubes.

13.5 Vortex, spin down, and incubate in the dark for about 2 min to optimize the reaction.

13.6 On the Home screen, press to select the Assay to perform (**dsDNA**)

13.7 Press Read standards & run samples to read new standards



13.8 When prompted, load the Qubit™ Flex Tube Strip containing Standard #1 into the sample chamber, then press Run standards. The reading takes ~3 seconds.

13.9 When prompted, load the Qubit™ Flex Tube Strip containing Standard #2 into the sample chamber, then press Run standards. The reading takes ~3 seconds.

13.10 If your calibration is successful, press Next to proceed to “Read samples” or begin in this step if it is already calibrated.

14 READ SAMPLES

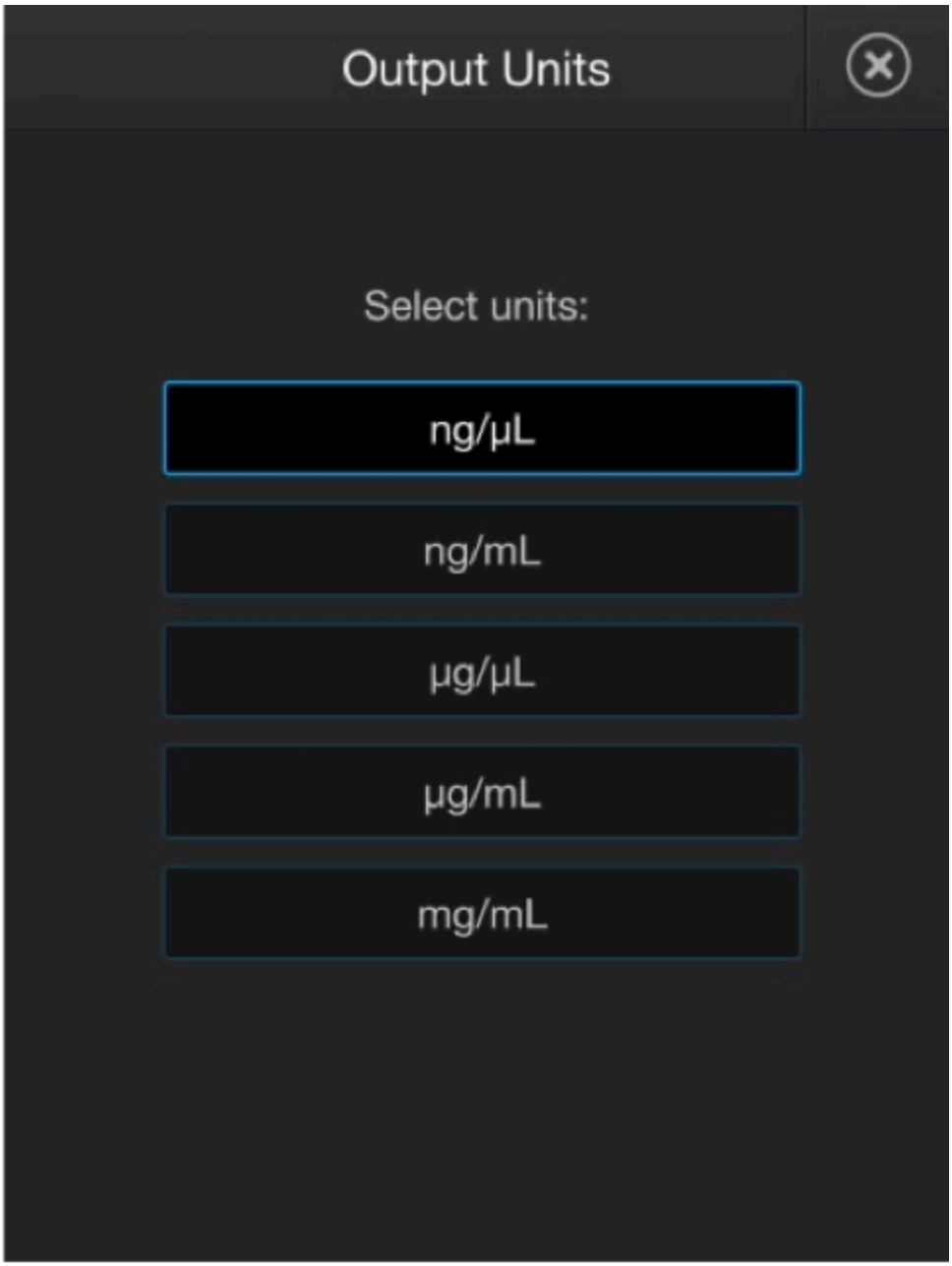
14.1 Add 198 µL or 199 µL of dsDNA Broad Range Working Solution to a 0.2 mL Qubit tube.

14.2 Add 2 µL or 1 µL of Sample thoroughly mixed (flick + spin down) in the Qubit tube (200 µL total amount)

14.3 Pulse-vortex and spin down. Incubate in dark for at least 2min.

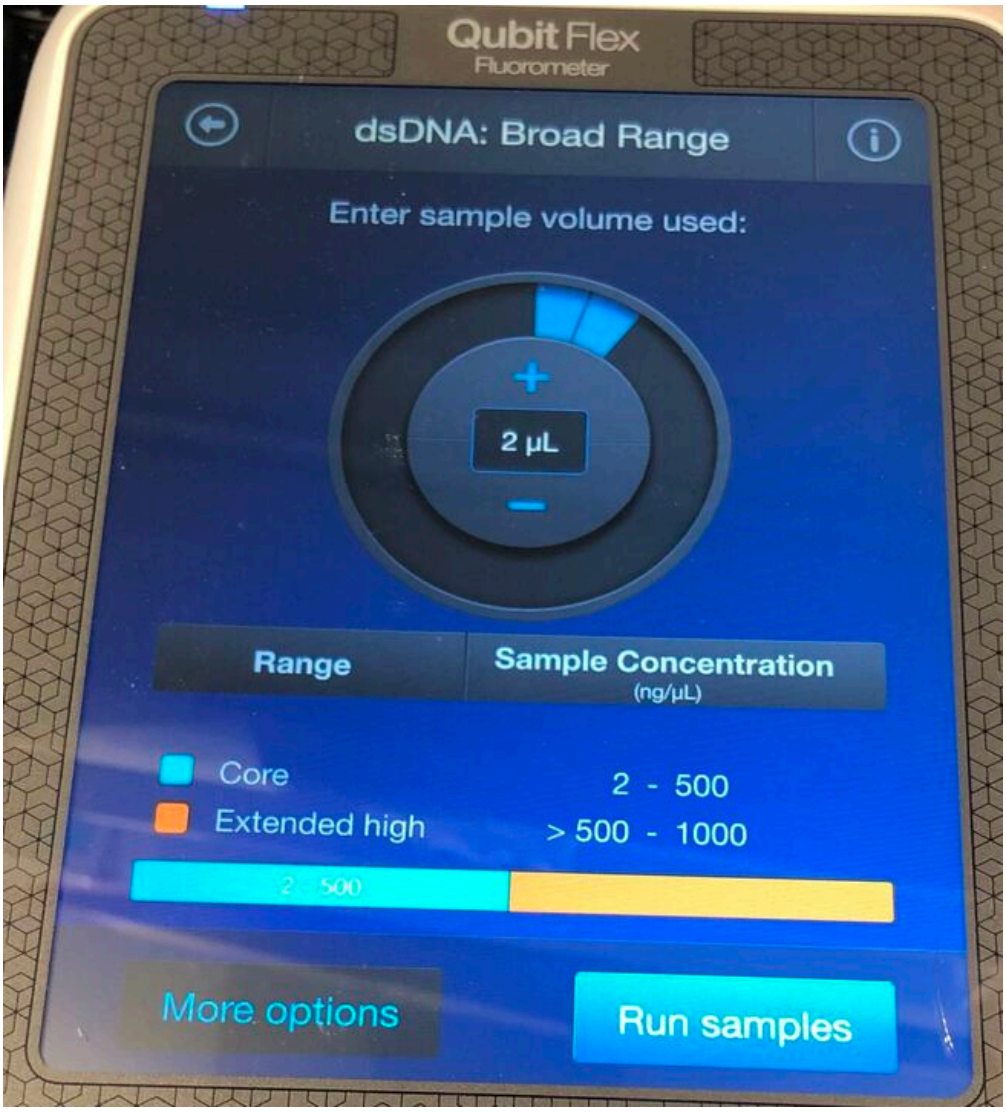
14.4 Load the tube strip containing the samples as shown. If you have fewer than 8 samples, press to deselect the tube positions that do not contain a sample.

14.5 Press Output sample units to open the Output Units screen, then select the desired units. Press Next to go to the Sample volume screen.



Select the units accordingly

14.6 In the Sample volume screen enter the sample volume used: it could be 1 µL or 2 µL .

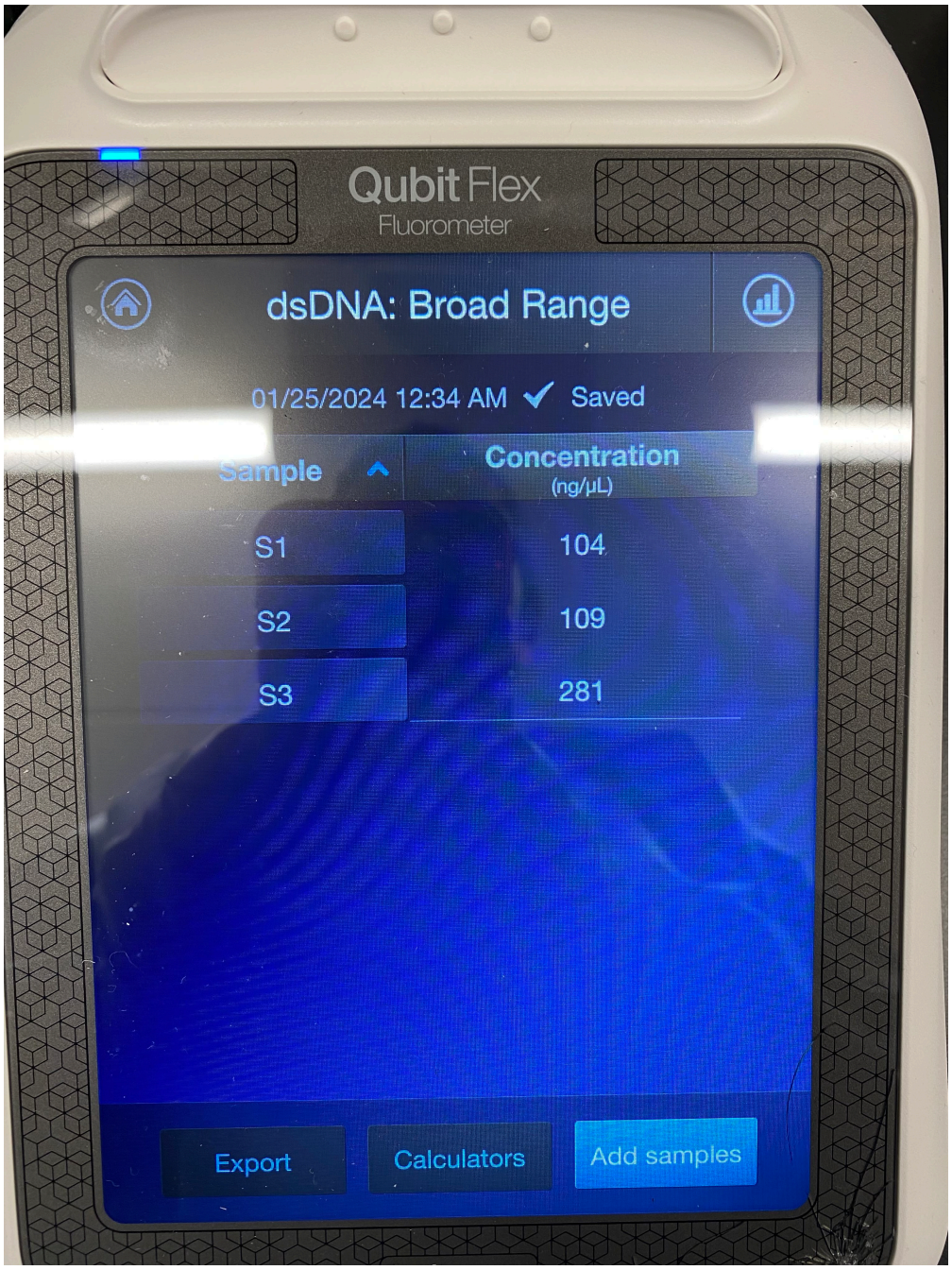


14.7 Press Run samples. The reading takes approximately 3 seconds.

14.8

Expected result

To display the results in list view, press the Graph button to hide the graph. The Results screen shows the concentration of each original sample in a list form, using the output units selected at the beginning of the assay.



On average, the Midi kit **yields about 20-30 micrograms of DNA** per sample. In this example, you can find this quantity by multiplying the Qubit concentration (104 nanograms per microliter) by the sample volume (550 microliters), then dividing the result by 1000. In this case, it equals 57.20

	Post-Ext. Qubit(ng/ul)	Post-Ext. Volume(ul)	Post-Ext. Amount (ug)
	104	550	57.20

Protocol references

QIAamp DNA Blood Midi Handbook
Thermofisher scientific