Standard Operating Procedure (SOP)	
SOP Title	Dried Blood Spot (DBS) Extraction: Vacuum methodology
SOP number	DBS02
SOP Version	2

1. SCOPE

The QIAamp DNA Investigator Kit uses well-established technology for purification of genomic and mitochondrial DNA from dried blood spotted onto filter paper. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes.
The procedure is designed to ensure that there is no sample-to-sample cross contamination. After sample lysis, the simple QIAamp DNA Investigator procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA. DNA is eluted in buffer ATE and is immediately ready for use in amplification reactions, or for storage at –20°C. The purified DNA is free of proteins, nucleases, and other inhibitors.

Principle and procedure.
The QIAamp DNA Investigator procedure consists of 4 steps:

- Lysis of blood spots: sample is lysed under denaturing conditions with Proteinase K.
- Binding DNA: DNA binds to the membrane and contaminants flow through.
- Wash: residual contaminants are washed away.
- DNA Elution: pure, concentrated DNA is eluted from the membrane.

2. MATERIALS REQUIRED:

2.1 REAGENTS & EQUIPMENT

DNA extraction kit	*Supplier*	*Cat. No.*
QIAamp DNA Investigator Kit	QIAGEN	56504
• QIAamp MinElute® Columns • Buffer AL* (33 mL) • Buffer ATL (50 mL) • Buffer AW1* - concentrate (19 mL) • Proteinase K (1.25 mL) • Buffer AW2† - concentrate (13 mL) • Buffer ATE (20 mL)		
Reagents	***Supplier***	***Cat. No.***
Ethanol – molecular grade (96–100%) ❄		
Materials	***Supplier***	***Cat. No.***
QIAvac 24 Plus manifold and Vacuum Pump	QIAGEN	19419
VacConnectors	QIAGEN	19407
96 well qPCR plate, skirted (AB2800)	Fisher Scientific	10032013
Microseal “A” seal	BIO-RAD	MSA 5001
Thermomixer / Heated orbital incubator		
Lo-bind Eppendorf tubes (1.5 mL)		
Centrifuge (Rotor suitable for Eppendorf tubes)		
Pipettes (1000, 200, 100, 20 and 1 µL)		
Filtered tips of various volumes		
Stripette gun & Stripettes (25 & 50mL)		

* Contains a guanidine salt. Not compatible with disinfectants containing bleach.
† Contains sodium azide as a preservative.
❄ Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone

2.2 PREPARATION OF BUFFERS

Preparing Buffer ATL
Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.

Preparing Buffer AW1
Add 25 mL ethanol (96–100%) to the bottle containing 19 mL Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year.
Note: Before starting the procedure, mix the reconstituted Buffer AW1 by shaking.

Preparing Buffer AW2
Add 30 mL ethanol (96–100%) to the bottle containing 13 mL Buffer AW2 concentrate. Reconstituted Buffer AW2 can be stored at room temperature (15–25°C) for up to 1 year.
Note: Before starting the procedure, mix the reconstituted Buffer AW2 by shaking.

Equilibrate the Buffer ATE for elution to room temperature (15–25°C).

If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.

2.3 QIAamp MinElute columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp MinElute columns to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp MinElute column. Pipet the sample into the QIAamp MinElute column without wetting the rim of the column.
- Always change pipette tips between liquid transfers. We recommend the use of filter tip pipette tips.
- Avoid touching the QIAamp MinElute column membrane with the pipette tip.
- After all pulse-vortexing steps, briefly centrifuge the micro-centrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.

3. METHODOLOGY

3.1 Preparing blood spots

Group the dried blood spots into batches of 94. Select a batch of 94 blood spots for extraction. Punch / cut the dried blood spots as described below:

3.1.01	Using a punch: ➢ Cut seven 4 mm diameter punches from a dried spot and place in a 1.5mL micro-centrifuge tube. Label micro-centrifuge tubes with sample ID. ➢ Taking a clean filter paper and soak in ethanol (absolute) and cut two punches to clean the punch. Dispose of punches.
3.1.02	Scissors or appropriate cutting device: ➢ Cut out a 0.5–2.5 cm^2 sample from the dried spot, and then cut it into smaller pieces. Transfer the pieces to a 1.5 mL micro-centrifuge tube and label micro-centrifuge tube with sample ID. ➢ Using an ethanol wipe clean the cutting implements. Dispose of wipes. Use a fresh wipe for each clean to reduce the chance of cross contamination.
3.1.03	Repeat for remainder of samples. Making sure that everything is cleaned between samples so that there is no cross contamination.

3.2	**LYSIS OF BLOOD SPOTS**
3.2.01	Add 300 µL of buffer ATL to each tube, close the lids and mix by pulse vortexing for 10 sec.
3.2.02	Add 30 µL of Proteinase K to each tube, close the lids and mix by pulse vortexing for 10 sec.
3.2.03	Pulse spin samples in a centrifuge at 1000rpm to remove drops from the inside of the lid.
3.2.04	Place the tubes in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at ~600 rpm for 17 h.
3.2.05	Pulse spin samples in a centrifuge at 1000rpm to remove drops from the inside of the lid.
At this point, the samples can be stored at -20°C until required for processing.	
3.2.06	Process the samples in batches of 24 from this point (assuming use of the 24 column manifold).
3.2.07	Add 300 µL Buffer AL, close the lid and mix. ➢ To ensure efficient lysis, it is essential that the sample and Buffer AL are thoroughly mixed to yield a homogeneous solution. ➢ A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation
3.2.08	Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.
3.2.09	Pulse spin samples in a centrifuge at 1000rpm to remove drops from the inside of the lid.
3.2.10	If solid particles are still visible, centrifuge at 6000 x g (8000 rpm) for 1 min.
3.3	**DNA BINDING TO MEMBRANE**
3.3.01	Carefully transfer each supernatant to a new 1.5 mL micro-centrifuge tube making sure that the filter paper is NOT transferred. Label micro-centrifuge tubes with sample ID.
3.3.02	Dispose of tube containing the used filter paper.
3.3.03	Add 150 µL ethanol (96–100%), close the lid, and mix thoroughly by pulse vortexing for 15 s. ➢ To ensure efficient binding, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.
3.3.04	Pulse spin samples in a centrifuge at 1000rpm to remove drops from the inside of the lid.

3.4 WASH	
3.4.01	Set up the vacuum pump and check the waste containers are empty.
3.4.02	Prepare the manifold and attach the VacConnectors. The VacConnectors are used to reduce the possibility of contamination.
3.4.03	Place the number of QIAamp MinElute columns required onto the manifold. Label each column with the appropriate sample ID.
3.4.04	Carefully transfer the supernatant to the allocated QIAamp MinElute column.
3.4.05	Start vacuum pump and run until the samples have been drawn through, then turn off. ➢ If the lysate has not completely passed through the membrane after vacuuming, then vacuum again for longer until the QIAamp MinElute column is empty.
3.4.06	Add 500 µL Buffer AW1 to the QIAamp MinElute column.
3.4.07	Start the vacuum pump and run until the samples have been drawn through then turn off.
3.4.08	Add 700 µL Buffer AW2 to the QIAamp MinElute column.
3.4.09	Start the vacuum pump and run until the samples have been drawn through then turn off.
3.4.10	Add 700 µL of ethanol (96–100%) to the QIAamp MinElute column
3.4.11	Start vacuum pump and run until the samples have been drawn through.
3.4.12	Keep the vacuum pump running to dry the membrane completely for ~10 min, then turn off. ➢ This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
3.4.13	Place the QIAamp MinElute column in a clean 1.5 mL micro-centrifuge tube
3.4.14	Carefully open the lid of the QIAamp MinElute column, and incubate at room temperature (15–25°C) for 10 min.
* The Buffer AW1 & AW2 flow-through contains guanethidine hydrochloride, which should be disposed of according to local regulations.	

3.5 DNA ELUTION	
3.5.01	Once membrane is dry apply 100 µL of Buffer ATE to the centre of the membrane **Important:** Ensure that the Buffer ATE has equilibrated to room-temperature. Dispense Buffer ATE onto the centre of the membrane to ensure complete elution of bound DNA
3.5.02	Close the lid and incubate at room temperature for 5 min
NOTE. Elution with small volumes increases the final DNA concentration in the eluate significantly, but reduces the overall DNA yield. Remember that the volume of eluate will be up to 5 µL less than the volume of elution solution applied to the column.	
3.5.03	Centrifuge at full speed (20,000 x g; 14,000 rpm) for 2 min.
3.5.04	For each sample check the volume and transfer to a 96 well plate. Make a note of the sample ID, location and volume of each sample.
3.5.05	Dispose of used columns and collection tubes.
3.5.06	Once the plate is complete then seal the plate with a Microseal “A” seal.
3.5.07	Samples can be stored at: • 4°C – short term storage (week) • -20°C/-80°C – long term storage (2 weeks plus)

APPENDIX 1: FURTHER KIT INFORMATION:

QIAamp MinElute columns should be stored at 2–8°C upon arrival and are stable under these conditions for at least one year after delivery. However, short-term storage (up to 4 weeks) at room temperature (15–25°C) does not affect their performance.
All buffers can be stored at room temperature (15–25°C) and are stable for at least one year after delivery.
The QIAamp DNA Investigator Kit contains a novel, ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least nine months after delivery when stored at room temperature in the dark. For storage longer than nine months store at 2–8°C.