Purification of Circulating Nucleic Acids from Plasma Protocol

Last Modified: March 31, 2020

SOP #: 2020.02.003 **Approved By:** **Trevor Pugh**

1.0 Purpose

Purification of circulating nucleic acids from plasma.

1.1 Equipment and Materials

Equipment	Materials
Water bath Heating Block Microcentrifuge Centrifuge QIAvac 24 Plus vacuum manifold QIAvac Connecting system Vacuum pump Vacvalves Luer Plugs Vortex	QIAGEN QIAamp Circulating Nucleic Acid Kit 100% Ethanol Wet ice 15 mL centrifuge tubes 50 mL centrifuge tubes 1.5 mL DNA Low Bind tubes

1.2 QIAamp Circulating Nucleic Acid Kit Special Storage

Materials
QIAGEN Mini Columns **- (4°)**
QIAGEN Proteinase K **- (4°)**

1.3 Resources

Worksheet:
smb://pughlab.srv.uhnresearch.ca/pughlab/Protocols/Worksheets_for_Current_Protocols/**2020.02.003_cfDNA_Extraction_QIAamp_Circulating_Nucleic_Acid_worksheet**.xlsx

2.0 Protocol

2.1 Sample Preparation

Notes:

- Before beginning, ensure that the number of samples being processed does not exceed the capacity of the water bath.
- Until DNA has been bound to extraction columns, work under Biological Safety Cabinet.
- In order to maximize yield, keep samples **on ice** until lysis.

1. Pre-cool a high-speed centrifuge with a 15 mL tube rotor adaptor.

2. Remove plasma samples from -80^{0}C freezer and allow sample to thaw at room temperature.

    - This may take 30-40 minutes if sample is in 2 mL tubes or 60 minutes if sample is in 15 mL tube. On Worksheet, make note of thawing time.
    - After taking out the 15 mL plasma tubes inspect carefully for any cracks. If the sample tube has any signs of damage, put in a separate 50 mL tube; let the plasma drain into the 50 mL tube as it thaws. Then transfer the whole sample to a new 15 mL tube for high speed spin.
    - Once thawed, keep plasma **on ice**.

3. Turn on water bath to 60°C.

4. If plasma is in 1.5 - 2 mL cryotubes, quick spin tubes in centrifuge and pipet plasma into a 15 mL Falcon Tube. *Be cautious of cross-contamination and sample swaps.*

    - After transferring the plasma, quick spin the 1.5 - 2 mL tubes again and collect any remaining sample.

5. Spin 15 mL Falcon Tubes with sample at 4°C 16,000 x g (brake speed set to 5) for 5 minutes.

NOTE: QIAamp kit column is designed to take up to 10 mL of plasma for optimal cfDNA yields; if working with larger volumes, multiple columns should be used

6. If plasma volume is greater than 10 mL for a given sample, split it in half and treat as two separate samples throughout the protocol. Ex: if a sample has 14 mL of plasma, split it into two samples of 7 mL. This would use double the consumables (columns, extender tubes, etc). Samples are named -1 and -2 to indicate replicates of identical samples.

2.2 Cell Lysis & Binding

1. Label one 50 mL tube per sample.

2. After centrifugation, transfer sample to labelled 50 mL tube, leaving any cryoprecipitates behind (up to 0.5 mL of plasma) and estimate final volume as accurately as possible. Record volume on Worksheet in order to calculate required reagent volumes.

NOTE: Use a serological pipette to measure plasma volume while transferring to 50 mL tubes.

3. Aliquot QIAGEN Proteinase K into sample tube. The amount of Pro K varies with the amount of plasma, where you add 0.1 mL of Pro K per mL of plasma.

4. Add Buffer ACL to 50 mL tube.

   - The amount of ACL varies with the amount of plasma, where you add 0.8 mL of ACL per mL of plasma.

5. Mix by pulse-vortexing for 30 s *(NOTE: it is crucial to vortex at least 30 s)*.

   - Make sure that a visible vortex forms in the tube.
   - If the volume of lysate is high and does not vortex properly, flip the tube on its top and quickly set the bottom back down into the centre of the vortexer to help a visible vortex form.
   - If necessary, one can seal the tube caps with parafilm before incubation.

6. Incubate in hot water bath at 60°C for **60 min**.

NOTE: Vacuum manifold can be set up during incubation.

7. Place the tube back on the lab bench, wipe down the outside of the tube with 70% ethanol.

8. Add Buffer ACB to the lysate in the 50 mL tube.

   - The amount of ACB varies with the amount of plasma, where you add 1.8 mL of ACB per mL of plasma.

9. Close the cap and mix thoroughly by pulse-vortexing for 30 s *(NOTE: it is crucial to vortex at least 30 s)*.

10. Incubate the lysate–Buffer ACB mixture in 50 mL tubes for **10 min** **on ice**.

2.3 Vacuum Manifold Setup

1. Wipe down manifold with 70% ethanol before use.

2. Attach black screw cap to right/end of manifold (hole closer to the bench surface). Can cover with parafilm if necessary to have better seal.

3. Attach quick-coupling female connector to left/front of manifold (hole up higher which will be attached to vacuum system).

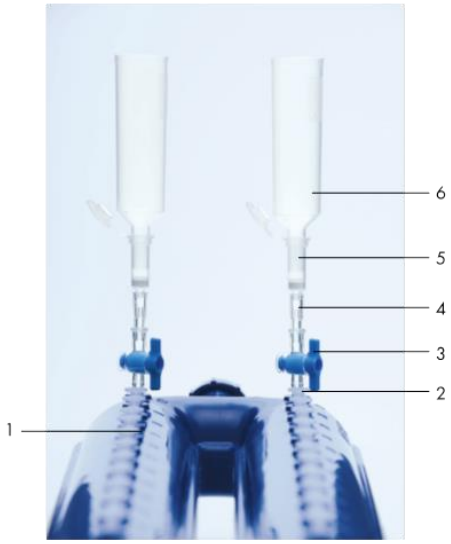
4. Connect the vacuum manifold to the vacuum pump.

5. Insert Luer plugs (2), which are reusable, into holes that are not being used for

filtration.

6. Insert VacValves (3), which are reusable, into the vacuum manifold slots to be used for plasma filtration. Insert the rest of the components into the VacValves in the following sequence: VacConnectors (4), QIAamp Mini column (5) and 20mL tube extender (6). See image below for details.

    - Ensure that the columns and tube extenders are well labelled and all components fit tightly into each other.
    - Ensure that when column caps are open, the sample label can be seen.



For further reference on setting up the vacuum system refer to Appendix 6.1.

7. Once vacuum system is setup, turn on pump and ensure it is working properly by reaching high pressure (800-1000 mbar) and can maintain pressure (~300 mbar).

2.4 Filtration

1. Carefully apply the lysate–Buffer ACB mixture into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. Make sure to maintain a pressure of at least 300 mbar.

    - If pressure is not correct, adjust level by carefully turning black knob at front of vacuum system (clockwise to increase pressure, counterclockwise to decrease).

2. Once 50 mL sample tubes appear empty, quick spin in centrifuge and pipet remaining solution into corresponding tube extender.

3. Turn on hot plate to 56^0 C .

4. When all lysates have been drawn through, close VacValves and remove and discard the tube extender (certain samples will take longer to pass through the column depending on volume and plasma composition; close VacValves after the sample finished passing through).

2.5 Wash DNA

1. Close the main vacuum control switch, open all the VacValves and apply 600 µl of Buffer ACW1 to the QIAamp Mini column; open the control switch and allow buffer to flow through all the columns simultaneously.

2. Close the main vacuum control switch and apply 750 µl of Buffer ACW2 to the QIAamp Mini column; open the control switch and allow buffer to flow through all the columns.

3. Close the main vacuum control switch and apply 750 µl of ethanol (96–100%) to the QIAamp Mini column; open the control switch and allow buffer to flow through all the columns. Close the control switch once all the buffer is through.

4. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 mL collection tube.

5. Centrifuge Mini column and collection tube at full speed for 3 min to dry the column membrane.

6. Place the QIAamp Mini Column into a new 1.5 mL collection tube. Open the lid and incubate the assembly at 56°C for **6 min** to dry the membrane completely.

2.6 Elute DNA

1. Place the QIAamp Mini column in a clean 1.5 mL DNA low bind elution tube.

2. Add 60-80 µl of Buffer AVE onto the center of the membrane for complete elution of DNA.

    - Can decrease elution volume to 50 µl or lower if expected yields are low to achieve higher concentration

3. Incubate for **5-10 minutes** at room temperature.

4. Spin Elution tubes with columns for 2 minutes at full speed.

5. Repeat another elution into same tube with 40-50 µl of Buffer AVE (or

adjust volumes as necessary), followed by RT incubation for **5 minutes** and 2-minute spin at max speed.

6. Quantify the yield using Qubit Fluorometer with **High Sensitivity** dsDNA Assay. Store the eluent at -80°C.

3.0 References

- **QIAamp Circulating Nucleic Acid Handbook:** https://www.qiagen.com/ca/resources/resourcedetail?id=0c4b31ab-f4fb-425f-99bf-10ab9538c061&lang=en
- **Qubit Operating Procedure:** https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf

4.0 Additional Resources

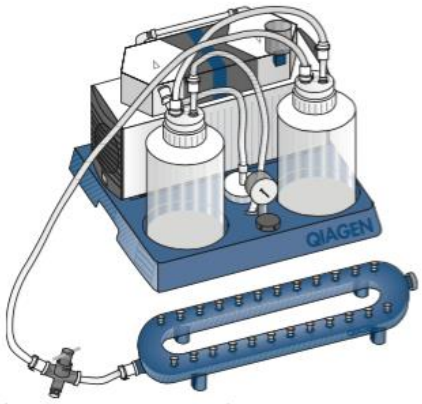
- Refer to **Plasma and Buffy Coat Separation** for Blood Tubes protocol (SOP # 2020.01.005) if sample is received as whole blood.
- Refer to **cfDNA & gDNA Library Preparation** using Molecular Barcoding and Dual Indexing (SOP# 2020.03.008) for next steps.

5.0 Modifications of Protocol

Date	Section	Initial & Change
August, 2016	All	EH; created the protocol
May 8, 2017	3.1-3.4	IC; edited format; added details to steps and introduced protocol modifications that took place over past year.
Jun 27, 2017	All	IC; updated several sections with new modifications
May 1, 2018		IC; updated sections with new protocol modifications
March 22, 2019	3	SP; Added details/tips
March 31, 2020	All	JW/PB/MC/SP; Updated and formatted

6.0 Appendix

6.1 QIAvac 24 Plus Vacuum & Manifold Setup



1. Plug in vacuum system
2. Connect vacuum manifold to vacuum pump
3. Close Manifold with black screw cap
4. Insert Luer plugs into all manifold plugs not being used for filtration. Try to have filtration holes evenly spread across the manifold
5. Set up as displayed in figure 3
6. Turn on vacuum and adjust pressure using black dial
7. Open VacValves and allow for filtration

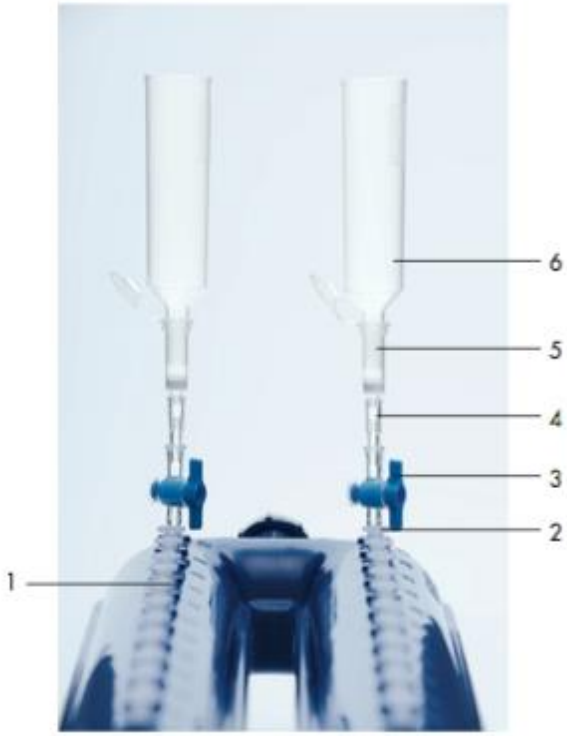


Figure 3. Setting up the QIAvac 24 Plus with QIAamp Mini columns using VacValves, VacConnectors, and Tube Extenders.

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus (closed with luer plug)
3. VacValve*
4. VacConnector
5. QIAamp Mini column
6. Tube Extender

* Must be purchased separately.