

April 2021

Supplementary Protocol

# Purification of genomic DNA from Whole Blood and Cultured Cells using the QIAamp® Fast DNA Tissue Kit

The QIAamp Fast DNA Tissue Kit (cat. no. 51404) can be stored at room temperature (15–25°C) for up to one year. When stored correctly, the QIAamp Fast DNA Tissue Kit is good until the expiration date printed on kit box lid.

**IMPORTANT:** Please consult the “Safety Information” and “Important Notes” sections in the *QIAamp Fast DNA Tissue Kit Handbook*, [www.qiagen.com/HB-1931](http://www.qiagen.com/HB-1931), before beginning this procedure.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Pipettes and disposable pipette tips with aerosol barriers (20–1000 µl)
- Isopropanol
- Ethanol (96–100%)\*
- Vortexer with adapter or microtube holder, e.g., the Horizontal (24) - Microtube Holder (cat. no. SI-H524) or Vertical (24) - Microtube Holder (cat. no. SI-V524) from Scientific Industries™ ([www.scientificindustries.com](http://www.scientificindustries.com)).
- Thermomixer at 56°C, for example, the Thermomixer Comfort (cat. no. 5355 000.011) and a thermoblock for 24 x 2 ml tubes (cat. no. 5362 000.019) from Eppendorf® ([www.eppendorf.com](http://www.eppendorf.com)).
- Benchtop centrifuge (with rotor for 2 ml tubes)
- **Optional:** TissueLyser II (QIAGEN, cat. no. 85300) with a TissueLyser II Adapter Set 2 x 24 (QIAGEN, cat. no. 69982), TissueLyser LT (QIAGEN, cat. no. 85600) with the TissueLyser LT Adapter for 12 tubes (QIAGEN, cat. no. 69980), or other bead-mill homogenizers.

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

## Important points before starting

- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Add isopropanol to Buffer MVL concentrate.
- Preheat a thermomixer or heating block to 56°C for use in step 4.
- Unless stated otherwise, all centrifugation steps should be performed at full speed (maximum 20,000 x *g*) for 30 seconds at room temperature in a conventional tabletop centrifuge.

## Procedure

1. A) Whole Blood: Pipet up to 200 µl whole blood into the Tissue Disruption Tube (supplied).

**Note:** If the sample volume is less than 200 µl, adjust volume to 200 µl with Buffer AVE (or PBS).

B) Cultured Cells: Centrifuge the maximum of  $5 \times 10^6$  cells for 5 min at 300 x *g* (190 rpm). Remove the supernatant completely and discard. Resuspend the pellet in 200 µl AVE (or PBS) and transfer the sample into the Tissue Disruption Tube (supplied).

2. Add the following buffers and enzymes: 40 µl VXL, 1 µl DX Reagent, 20 µl Proteinase K, 4 µl RNase A (100 mg/ml). Tightly cap the lid.

**Optional:** When processing multiple samples, you may prepare a master digestion buffer mix.

3. Homogenize using one of the following two options:

3a. Vortex-Genie® 2 with appropriate 2 ml tube adapter: full speed for 1 min

3b. TissueLyser LT: 40 Hz for 30 s

4. Incubate in a thermomixer (without shaking) or heating block for 10 min at 56°C.

5. Add 265 µl Buffer MVL and mix by pipetting or vortexing.

6. Apply the mixture from step 5 to the QIAamp Mini spin column and centrifuge for 1 min. Place the spin column into a new 2 ml collection tube (supplied).

7. Add 500 µl Buffer AW1 to the spin column and centrifuge. Place the spin column into a new 2 ml collection tube (supplied).

8. Add 500 µl Buffer AW2 to the spin column and centrifuge. Place the spin column into a new 2 ml collection tube (supplied).

9. Centrifuge for 2 min. Place the spin column into a clean 1.5 ml microcentrifuge tube (supplied).

10. Add 50–100 µl ATE directly onto the spin column membrane, incubate at room temperature for 1 min, and then centrifuge for 1 min.

**Optional:** Repeat step 10 for increased yield.

## Document revision history

Date	Changes
04/2021	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

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