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## Protocol 002: DNA extraction from Blood using QIAamp DNA Mini Kit (Qiagen)

Version Number	Written by	Reviewed by	Approved by
1.0			Prof. Samuel Wanji

### Principle:

QIAamp DNA Mini Kit is designed for rapid purification of an average of 6 µg of total DNA (e.g., genomic, viral, mitochondrial) from 200 µl of whole human blood, and up to 50 µg of DNA from 200 µl of buffy coat, 5 x 10<sup>6</sup> lymphocytes, or cultured cells that have a normal set of chromosomes. The procedure is suitable for use with whole blood treated with citrate, heparin, or EDTA;\* buffy coat; lymphocytes; plasma; serum; and body fluids. Samples may be either fresh or frozen. For larger volumes of whole blood or cultured cells, we recommend using QIAamp DNA Blood Midi or Maxi Kits. Furthermore, the QIAamp DNA Mini Kit performs all the functions of the QIAamp DNA Blood Mini Kit, and also allows rapid purification of DNA from solid tissue. On average, up to 30 µg of DNA can be purified from 25 mg of various human tissues.

### Requirements

Equipment	Consumables	Reagents
<ul style="list-style-type: none"> <li>• Refrigerator</li> <li>• Freezer (-20<sup>0</sup>C)</li> <li>• Micro pipettes</li> <li>• Microcentrifuge</li> <li>• Timer</li> <li>• Vortex mixer</li> <li>• Water bath or heating block</li> <li>• Equipment for mechanical disruption (For tissues)</li> </ul>	<ul style="list-style-type: none"> <li>• Tissue paper</li> <li>• Disposable gloves</li> <li>• 70% Ethanol</li> <li>• 1.5ml microcentrifuge tubes</li> <li>• 2 ml microcentrifuge tubes</li> <li>• Sterile, nuclease-free filter pipette tips</li> <li>• Ethanol (96-100%)</li> <li>• Marker pens</li> <li>• Ethanol resistant pens</li> </ul>	<p><b>QIAamp DNA Minikit (Qiagen)</b></p> <ul style="list-style-type: none"> <li>• QIAamp Mini Spin columns</li> <li>• Collection tubes (2ml)</li> <li>• Buffer ATL</li> <li>• Buffer AL</li> <li>• Buffer AW1 (Concentrate)</li> <li>• Buffer AW2 (Concentrate)</li> <li>• Buffer AE</li> <li>• PBS (For some samples)</li> </ul>

### Storage of reagents

All QIAamp DNA Mini Kit reagents should be stored at room temperature  
SOP DNA extraction from blood

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### **Extraction procedure for whole blood**

- When using a new QIAamp DNA Mini Kit add alcohol to buffers AW1 and AW2 as indicated on the bottles. Note the date on each bottle in alcohol resistant ink.
  - All centrifugation steps should be carried out at room temperature.
1. Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.
  2. Add 200 µl sample to the microcentrifuge tube. Use up to 200 µl whole blood, plasma, or serum. If the sample volume is less than 200 µl, add the appropriate volume of PBS.
  3. Add 200 µl Buffer AL to the sample, mix by pulse-vortexing for 15 s.
  4. Incubate at 56°C for 10 min.
  5. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
  6. Add 200 µl ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid
  7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
  8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
  9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

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10. **Recommended:** Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
11. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200  $\mu$ l Buffer AE or distilled water. Incubate at room temperature for 5-10 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.