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# Extraction of DNA from 2 ml blood samples using Flexigene Kit (QIAGEN)

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**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** extraction of dna, ml blood sample, extraction, using flexigene kit, dna, flexigene kit, qiagen

## Materials

MATERIALS

- Isopropanol
- Ethanol 70%
- Buffer FG1 (lysis buffer) **Qiagen Catalog #**51206
- Buffer FG2 (denaturation buffer) **Qiagen Catalog #**51206
- Buffer FG3 (hydration buffer) **Qiagen Catalog #**51206
- Qiagen Protease **Qiagen Catalog #**51206

STEP MATERIALS

- Buffer FG1 (lysis buffer) **Qiagen Catalog #**51206
- Buffer FG2 (denaturation buffer) **Qiagen Catalog #**51206
- Qiagen Protease **Qiagen Catalog #**51206
- Isopropanol
- Ethanol 70%
- Buffer FG3 (hydration buffer) **Qiagen Catalog #**51206
- Buffer FG1 (lysis buffer) **Qiagen Catalog #**51206
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- Isopropanol
- Ethanol 70%
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## Protocol materials

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- Isopropanol
- Ethanol 70%
- Buffer FG3 (hydration buffer) **Qiagen Catalog** #51206

## Troubleshooting

## Safety warnings

Protease mixture should be prepared not more than 1 hour before use.
All centrifugation steps should be carried out at room temperature in a swing-out rotor using conical tubes.

## Before start

Resuspend the lyophilized QIAGEN Protease in 1.4 ml of Buffer FG3 (hydration buffer) and store at 2–8°C or in aliquots at –20°C

Frozen blood should be thawed in a 37°C water bath

For every 1 ml of blood, mix together 500 µl Buffer FG2 (denaturation buffer) and 5 µl reconstituted QIAGEN Protease

## Mix the lysis buffer

1 Pipet 5 ml Buffer FG1 into a 10 ml centrifuge tube. Add 2 ml whole blood and mix by inverting the tube 5 times.

2 mL whole blood sample

5 mL Buffer FG1

Buffer FG1 (lysis buffer) **Qiagen Catalog #**51206

2 Centrifuge for 5 min at 2000 x g in a swing-out rotor.

00:05:00 Centrifugation

3 Discard the supernatant and leave the tube inverted on a clean sheet of absorbent paper for 2 min, taking care that the pellet remains in the tube.

Note

In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of supernatant from the rim and sides of the tube onto the pellet.

00:02:00 leave tube inverted on a sheet of absorbent paper

## Add denaturation buffer

4 Add 1 ml Buffer FG2/QIAGEN Protease, close the tube, and vortex immediately until the pellet is completely homogenized. Inspect the tube to check that homogenization is complete.

Note

When processing multiple samples, vortex each tube immediately after addition of Buffer FG2/QIAGEN Protease. Do not wait until buffer has been added to all samples before vortexing.
Usually 3–4 pulses of high-speed vortexing for 5 s each are sufficient to homogenize the pellet. However, traces of pellet with a jelly-like consistency (often barely visible) may remain. If these traces are seen, add a further 300 µl Buffer FG2 and vortex again.

1 mL Buffer FG2/QIAGEN Protease

Buffer FG2 (denaturation buffer) **Qiagen Catalog #**51206

Qiagen Protease **Qiagen Catalog #**51206

## Incubate

5 Invert the tube 3 times, place it in a heating block or water bath, and incubate at 65°C for 10 min.

Note

The sample changes color from red to olive green, indicating protein digestion.

00:10:00 Incubation at 65°C

## Add isopropanol and mix

6 Add 1 ml isopropanol (100%) and mix thoroughly by inversion until the DNA precipitate becomes visible as threads or a clump.

Note

Complete mixing with isopropanol is vital to precipitate the DNA and must be checked by inspection. For samples with very low white blood cell 2 counts, in which the DNA may not be visible, invert the tube at least 20 times.

Isopropanol

7 Centrifuge for 3 min at 2000 x g.

00:03:00 Centrifugation

Note

If the resulting pellets are loose, centrifugation can be prolonged or a higher g-force can be used.

8 Discard the supernatant and briefly invert the tube onto a clean piece of absorbent paper, taking care that the pellet remains in the tube.

Note

In rare cases the pellet may be loose, so pour slowly. If the white blood cell count of the sample was sufficiently high, the DNA should be visible as a small white pellet.

## Add 70% ethanol

9 Add 1 ml 70% ethanol and vortex for 5 s.

00:00:05 Vortex

1 mL 70% Ethanol

Ethanol 70%

10 Centrifuge for 3 min at 2000 x g.

00:03:00 Centrifugation

Note

If the resulting pellets are loose, centrifugation can be prolonged or a higher g-force can be used.

11 Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for at least 5 min, taking care that the pellet remains in the tube.

Note

In rare cases the pellet may be loose, so pour slowly. Inverting the tube onto absorbent paper minimizes backflow of ethanol from the rim and sides of the tube onto the pellet.

00:05:00 leave the tube inverted on a piece of absorbent paper

## Air-dry pellet and resuspend DNA

12 Air-dry the DNA pellet until all the liquid has evaporated (at least 5 min).

Note

Avoid over-drying the DNA pellet, since over-dried DNA is very difficult to dissolve.

00:05:00 air-dry the pellet

13 Add 200 µl Buffer FG3, vortex for 5 s at low speed, and dissolve the DNA by incubating for 1 h at 65°C in a heating block or water bath.

01:00:00 Incubation at 65°C

Note

If the DNA is not completely dissolved, incubate the solution overnight at room temperature. If a reduced volume of Buffer FG3 is used, the incubation time may need to be prolonged.

Buffer FG3 (hydration buffer) **Qiagen Catalog #**51206