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

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Abstract

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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

Materials

- Isopropanol by Contributed by users
- Ethanol 70% by Contributed by users
- Buffer FG1 (lysis buffer) 51206 by Qiagen
- Buffer FG2 (denaturation buffer) 51206 by Qiagen
- Buffer FG3 (hydration buffer) 51206 by Qiagen
- Qiagen Protease 51206 by Qiagen

Protocol

Mix the lysis buffer

Step 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.

AMOUNT
2 ml : whole blood sample
AMOUNT
5 ml : Buffer FG1
REAGENTS
Buffer FG1 (lysis buffer) 51206 by Qiagen

Step 2.

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

DURATION
00:05:00 : Centrifugation

Step 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.

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

NOTES

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.

Add denaturation buffer

Step 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.

AMOUNT
1 ml : Buffer FG2/QIAGEN Protease
REAGENTS
Buffer FG2 (denaturation buffer) 51206 by Qiagen
Qiagen Protease 51206 by Qiagen

NOTES

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.

Incubate

Step 5.

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

DURATION
00:10:00 : Incubation at 65°C

NOTES

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

Add isopropanol and mix

Step 6.

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

REAGENTS
Isopropanol by Contributed by users

NOTES

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.

Step 7.

Centrifuge for 3 min at 2000 x g.

DURATION
00:03:00 : Centrifugation

NOTES

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

Step 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.

NOTES

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

Step 9.

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

AMOUNT
1 ml : 70% Ethanol

REAGENTS
Ethanol 70% by Contributed by users

DURATION
00:00:05 : Vortex

Step 10.

Centrifuge for 3 min at 2000 x g.

DURATION
00:03:00 : Centrifugation

NOTES

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

Step 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.

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

NOTES

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.

Air-dry pellet and resuspend DNA

Step 12.

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

DURATION
00:05:00 : air-dry the pellet

NOTES

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

Step 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.

REAGENTS

Buffer FG3 (hydration buffer) 51206 by Qiagen

DURATION

01:00:00 : Incubation at 65°C

NOTES

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.

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.