November 2023

FlexiGene® DNA Handbook

For purification of DNA from human whole blood, buffy coat, and cultured cells

Sample to Insight

Contents

Kit Contents 3

Shipping and Storage 4

Intended Use 4

Safety Information 5

Quality Control 6

Introduction 7

Principle and procedure 7

Equipment and Reagents to be Supplied by User 9

Important Notes 10

Starting material 10

Determination of concentration, yield, and purity 13

Optimized handling for higher throughputs 13

Protocol: Isolation of DNA from 100–500 µL Whole Blood 14

Protocol: Isolation of DNA from 1–3 mL Whole Blood 18

Protocol: Isolation of DNA from 4–14 mL Whole Blood 22

Protocol: Isolation of DNA from 20 mL Whole Blood 26

Protocol: Isolation of DNA from 100–500 µL Buffy Coat 30

Protocol: Isolation of DNA from 1–2 mL Buffy Coat 34

Protocol: Isolation of DNA from 1–2 x 10^6 Cultured Cells 38

Troubleshooting Guide 42

Ordering Information 45

Document Revision History 46

Kit Contents

FlexiGene DNA Kit	
Catalog no.	**51206**
Volume of blood that can be processed per kit	**250 mL**
Buffer FG1 (lysis buffer)	3 x 220 mL
Buffer FG2* (denaturation buffer)	5 x 30 mL
Buffer FG3 (hydration buffer)	5 x 50 mL
QIAGEN Protease	1 vial†
Handbook	1

* Contains chaotropic salt, which is harmful. Not compatible with disinfectants containing bleach. See page 5 for Safety Information.

† Resuspension volume 1.4 mL

Shipping and Storage

All buffers and reagents can be stored at room temperature (15–25°C) for up to 24 months without reduction in performance.

Lyophilized QIAGEN Protease can be stored at room temperature (15–25°C) for up to 24 months without reduction in performance. For storage longer than 24 months or if ambient temperatures frequently exceed 25°C, QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for 2 months when stored at 2–8°C. Incubating the QIAGEN Protease stock solution at room temperature for prolonged periods of time should be avoided. Storage at –30°C to –15°C will prolong its life, but repeated freezing and thawing should be avoided. Dividing the solution into aliquots and storage at –30°C to –15°C is recommended.

Intended Use

The FlexiGene DNA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The sample-preparation waste contains guanidine hydrochloride from Buffer FG2, which can form highly reactive compounds when combined with bleach.

If liquid containing Buffer FG2 is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of FlexiGene DNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

FlexiGene DNA Kits provide a rapid and simple method for purification of DNA from human whole blood, buffy coat, and cultured cells. The procedure can be scaled up or down, allowing purification from variable amounts of starting material. Purification is performed in a single tube, which reduces waste and minimizes the risk of sample mix-up. The use of toxic organic reagents is completely avoided. FlexiGene DNA Kits provide good yields of high-purity DNA that is free from contaminants or inhibitors. The purified DNA performs well in a range of downstream applications including PCR-based techniques, restriction digestion, blotting, and sequencing, or can be safely stored at 2–8°C or –30°C to –15°C.

Principle and procedure

Lysis buffer is added to the sample (see Figure 1, next page). Cell nuclei and mitochondria are pelleted by centrifugation. The pellet is resuspended and incubated in denaturation buffer, which contains a chaotropic salt, and QIAGEN Protease. This step efficiently removes contaminants such as proteins. DNA is precipitated by addition of isopropanol, recovered by centrifugation, washed in 70% ethanol, dried, and resuspended in hydration buffer (10 mM Tris·Cl, pH 8.5).

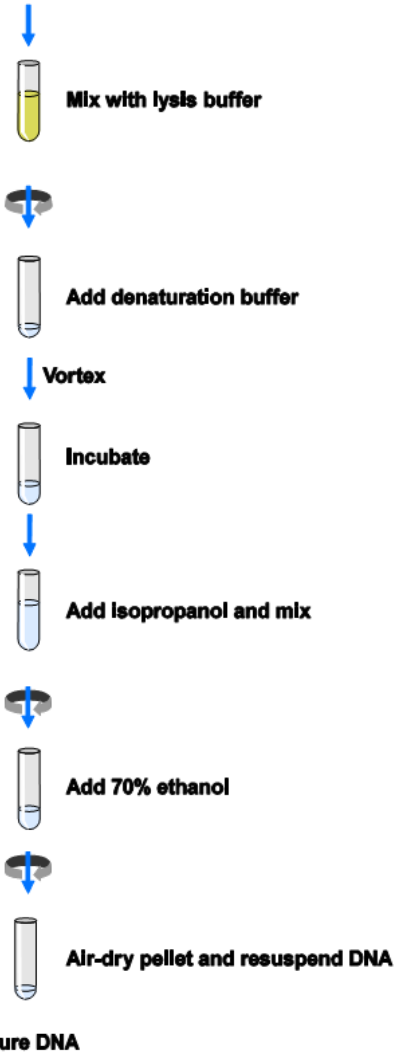

Figure 1. The FlexiGene Procedure: blood/buffy coat/cultured cells.

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.

- 100% isopropanol
- 70% ethanol
- Pipettes, and sterile DNase-free pipette tips with aerosol barrier
- For blood volumes <0.6 mL: 1.5 mL or 2 mL microcentrifuge tubes that tolerate 10,000 x *g* and a microcentrifuge capable of attaining 10,000 x *g*, equipped with a fixed-angle rotor
- For blood volumes ≥0.6 mL: 15 mL and 20 mL conical centrifuge tubes that tolerate 2000 x *g* and a centrifuge capable of attaining 2000 x *g*, equipped with a swing-out rotor
- Heating block or water bath
- Vortex mixer

Important Notes

Starting material

FlexiGene DNA Kits can be used with the following amounts of starting material.

Sample	Amount of starting material
Blood	0.1–20 mL
Buffy coat	0.1–2 mL
Cultured cells	≤2 x 10^6 cells

All samples may be either fresh or frozen. Frozen samples should be thawed quickly in a 37°C water bath with mild agitation before beginning the procedure.

Storage of blood samples

Whole blood samples treated with EDTA, citrate, or heparin can be used, and may be either fresh or frozen. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples yield better results.

- For short-term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.
- For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store at –70°C.

Yield and size of purified DNA

DNA yields depend on the number of nucleated cells present. Yields from whole blood may vary widely, since white blood cell counts can differ as much as tenfold. Table 1 shows typical yields obtained from different samples.

Table 1. DNA yields obtained from different sample types using the FlexiGene procedure

Sample	Volume	DNA yield (µg)
Blood*	0.3 mL	11–14
Blood*	2 mL	75–90
Blood*	10 mL	380–460
Buffy coat†	0.5 mL	110–130
HeLa cells	10^6 cells	25–35

* Whole blood with normal white blood cell counts (7×10^6 cells/mL blood).
† Buffy coat with 3×10^7 white blood cells/mL.

DNA size

The purified DNA is up to 150 kb in size (Figure 2). DNA of this length is suitable for all downstream applications, including Southern blotting, and can be amplified very efficiently (see Figure 3).

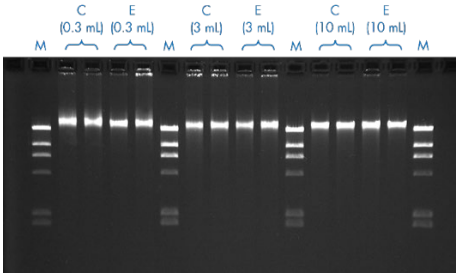

Figure 2. High-quality genomic DNA. Genomic DNA isolated from whole blood taken from 4 donors. Starting blood volumes are indicated above the lanes. **[C]** Blood treated with sodium citrate. **[E]** Blood treated with EDTA. **[M]** Markers.

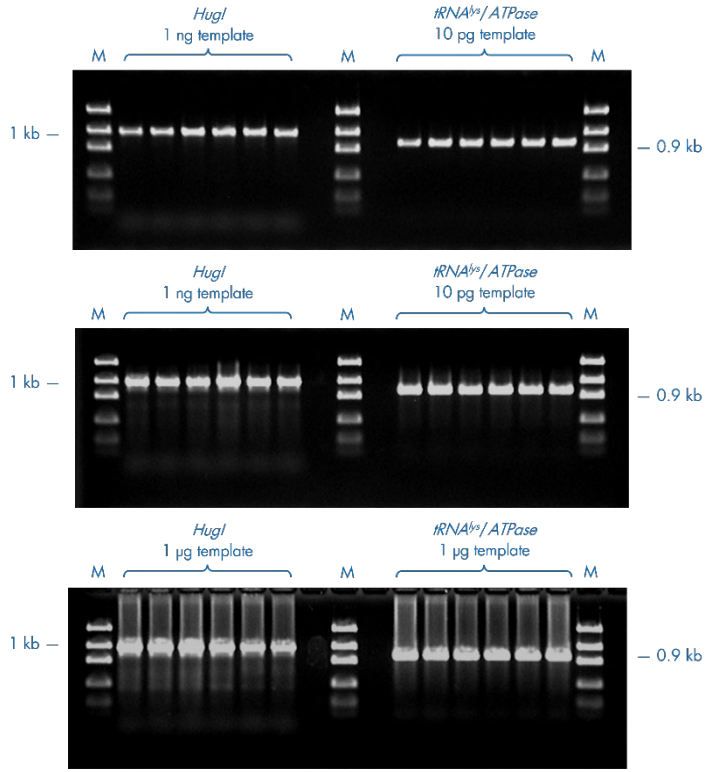

Figure 3. Efficient downstream PCR analysis. Variable amounts of DNA template were used to amplify the single-copy *HugI* gene and a mitochondrial target (*tRNA^{lys}/ATPase*). *Each sample was analyzed 6 times, and reproducible results were achieved.* **[M]** *markers.*

Determination of concentration, yield, and purity

DNA yield is determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Use hydration buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 nm and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9. The FlexiGene procedure typically yields DNA with an A_{260}/A_{280} ratio >1.7, indicating that the DNA is free of protein contamination.

Optimized handling for higher throughputs

- Use dedicated centrifuges equipped with suitable rotors for the different protocols.
  QIAGEN recommends use of the following:
  - For protocols requiring 1.5 mL or 2 mL microcentrifuge tubes: Eppendorf centrifuge 5417C with 30-well fixed-angle rotor FA-45-30-11 (**www.eppendorf.de**)
  - For protocols requiring 15 mL centrifuge tubes: Sigma centrifuge 4-16S or 4-16KS with swing-out rotor (cat. no. 11150) and 4 buckets (cat. no. 13236) for 12 tubes each (**www.sigma-zentrifugen.de**)
  - For protocols requiring 50 mL centrifuge tubes: Sigma centrifuge 4-16S or 4-16KS with swing-out rotor (cat. no. 11150) and 4 buckets (cat. no. 13235) for 7 tubes each (**www.sigma-zentrifugen.de**)
- Divide Buffer FG1 (lysis buffer) into aliquots and store these in appropriate purification tubes.
- Use hand and/or bottletop dispensers to dispense reagents.
- Invert multiple tubes simultaneously, not individually, by clamping tubes into a rack with an empty rack, grasping both racks, and inverting them together.
- Use a multi-tube vortexer or a vortexer equipped with a platform head.
- Use a shaker-incubator for rehydration of purified DNA in Buffer FG3 (hydration buffer).

Protocol: Isolation of DNA from 100–500 µL Whole Blood

Important points before starting

- The buffer volumes given in the following protocol are suitable for isolation of DNA from 300 µL samples of whole blood. The protocol can be adapted for DNA isolation from blood samples of 100–500 µL by scaling reagent volumes down or up in proportion to the volume of blood used (see Table 2). However, although the volume of Buffer FG3 used to dissolve the DNA in step 13 should be reduced to 100 µL for a starting blood volume of 100 µL, it should remain at 200 µL for starting blood volumes of 200 µL or more.

Table 2. Reagent volumes required for processing 100–500 µL blood samples

	Blood volume (µL)				
	100	**200**	**300**	**400**	**500**
Buffer FG1	250	500	750	1000	1250
Buffer FG2/QIAGEN Protease	50	100	150	200	250
100% isopropanol	50	100	150	200	250
70% ethanol	50	100	150	200	250
Buffer FG3	100	200	200	200	200

- All centrifugation steps should be carried out at room temperature in a fixed angle rotor.
- For DNA isolation from blood samples of ≥300 µL to 500 µL, 2 mL centrifuge tubes must be used.

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Calculate the total volume of blood to be processed. For every 1 mL of blood, mix together 500 µL Buffer FG2 (denaturation buffer) and 5 µL reconstituted QIAGEN Protease (see Table 3). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.

Table 3. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	100	300	500	1000	3000	5000	6000
Volume of Buffer FG2 (µL)	50	150	250	500	1500	2500	3000
Volume of QIAGEN Protease (µL)	0.5	1.5	2.5	5	15	25	30

Procedure

1. Pipette 750 µL Buffer FG1 into a 1.5 mL centrifuge tube. Add 300 µL whole blood and mix by inverting the tube 5 times
2. Centrifuge for 20 s at 10,000 x *g* in a fixed-angle rotor
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.

4. Add 150 µL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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 another 30 µL of Buffer FG2 and vortex again.

5. Centrifuge the tube briefly (3–5 s), place it in a heating block or water bath, and incubate at 65°C for 5 min.

6. Add 150 µL 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 counts, in which the DNA may not be visible, invert the tube at least 20 times.

7. Centrifuge for 3 min at 10,000 x *g*.

   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.

9. Add 150 µL 70% ethanol and vortex for 5 s.

10. Centrifuge for 3 min at 10,000 x *g*.

    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.

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.

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.

    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.

Protocol: Isolation of DNA from 1–3 mL Whole Blood

Important points before starting

- The buffer volumes given in the following protocol are suitable for isolation of DNA from 3 mL samples of whole blood. The protocol can be adapted for DNA isolation from blood samples of 1–3 mL by scaling down reagent volumes in proportion to the volume of blood used (see Table 4). However, the volume of Buffer FG3 used to dissolve the DNA in step 13 should not be reduced to less than 200 µL.
- All centrifugation steps should be carried out at room temperature in a swing-out rotor using conical tubes.

Table 4. Reagent volumes required for processing 1–3 mL blood samples

	Blood volume (mL)		
Sample protocol	**1.0**	**2.0**	**3.0**
Buffer FG1	2.5	5.0	7.5
Buffer FG2/QIAGEN Protease	0.5	1.0	1.5
100% isopropanol	0.5	1.0	1.5
70% ethanol	0.5	1.0	1.5
Buffer FG3	0.2	0.2	0.3

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit.
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.

- Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Calculate the total volume of blood to be processed. For every 1 mL of blood, mix together 500 µL Buffer FG2 (denaturation buffer) and 5 µL reconstituted QIAGEN Protease (see Table 5). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.

Table 5. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	1	3	6	12	18	36
Volume of Buffer FG2 (µL)	0.5	1.5	3	6	9	18
Volume of QIAGEN Protease (µL)	5	15	30	60	90	180

Procedure

1. Pipette 7.5 mL Buffer FG1 into a 15 mL centrifuge tube. Add 3 mL whole blood and mix by inverting the tube 5 times.
2. Centrifuge for 5 min at 2000 x *g* in a swing-out rotor.
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.
4. Add 1.5 mL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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.

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.

6. Add 1.5 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 counts, in which the DNA may not be visible, invert the tube at least 20 times.

7. Centrifuge for 3 min at 2000 x *g*.

   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.

9. Add 1.5 mL 70% ethanol and vortex for 5 s.

10. Centrifuge for 3 min at 2000 x *g*.

    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.

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.

13. Add 300 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.

    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.

Protocol: Isolation of DNA from 4–14 mL Whole Blood

Important points before starting

- The buffer volumes given in the following protocol are suitable for isolation of DNA from 10 mL samples of whole blood. The protocol can be adapted for DNA isolation from blood samples of 4–14 mL by scaling reagent volumes down or up in proportion to the volume of blood used (see Table 6). However, for blood volumes >10 mL, only the volume of Buffer FG1 needs to be increased: all other reagent volumes remain at the levels used for 10 mL blood.

Table 6. Reagent volumes required for processing 4–14 mL blood samples

	Blood volume (mL)										
Sample protocol	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**	**9.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.0**
Buffer FG1	10.0	12.5	15.0	17.5	10.0	22.5	25.0	27.5	30.0	32.5	35.0
Buffer FG2/QIAGEN Protease	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.0	5.0	5.0	5.0
100% isopropanol	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.0	5.0	5.0	5.0
70% ethanol	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.0	5.0	5.0	5.0
Buffer FG3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.0	1.0	1.0	1.0

- All centrifugation steps should be carried out at room temperature in a swing-out rotor using conical tubes.

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Calculate the total volume of blood to be processed. For every 4 mL of blood, mix together 2 mL Buffer FG2 (denaturation buffer) and 20 µL reconstituted QIAGEN Protease (see Table 7). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.

Table 7. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	4	10	14	24	48	60	84	120	168
Volume of Buffer FG2 (µL)	2	5	7	12	24	30	42	60	84
Volume of QIAGEN Protease (µL)	20	50	70	120	240	300	420	600	840

Procedure

1. Pipette 25 mL Buffer FG1 into a 50 mL centrifuge tube. Add 10 mL whole blood and mix by inverting the tube 5 times
2. Centrifuge for 5 min at 2000 x *g* in a swing-out rotor
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.

4. Add 5 mL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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.

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.

6. Add 5 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 counts, in which the DNA may not be visible, invert the tube at least 20 times.

7. Centrifuge for 3 min at 2000 x *g*.

   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.

9. Add 5 mL 70% ethanol and vortex for 5 s.
10. Centrifuge for 3 min at 2000 x *g*.

    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.

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.

13. Add 1 mL 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.

    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.

Protocol: Isolation of DNA from 20 mL Whole Blood

Important point before starting

- All centrifugation steps should be carried out at room temperature in a swing-out rotor using conical tubes.

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit.
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Frozen blood should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Calculate the total volume of blood to be processed. For every 20 mL of blood, mix together 5 mL Buffer FG2 (denaturation buffer) and 50 µL reconstituted QIAGEN Protease (see Table 8). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 8 and 16.

Table 8. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	20	120	240
Volume of Buffer FG2 (µL)	5	30	60
Volume of QIAGEN Protease (µL)	50	300	600

Procedure

1. Pipette 25 mL Buffer FG1 into a 50 mL centrifuge tube. Add 10 mL whole blood and mix by inverting the tube 5 times
2. Centrifuge for 5 min at 2000 x *g* in a swing-out rotor
3. Discard the supernatant, taking care that the pellet remains in the tube.

   Note: In rare cases the pellet may be loose, so pour slowly.
4. Pipette 25 mL Buffer FG1 into a 50 mL centrifuge tube. Add another 10 mL whole blood and mix by inverting the tube 5 times
5. Centrifuge for 5 min at 2000 x *g* in a swing-out rotor
6. 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.
7. Add 5 mL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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 1 mL Buffer FG2 and vortex again.

8. 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.

9. Add 5 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 counts, in which the DNA may not be visible, invert the tube at least 20 times.

10. Centrifuge for 3 min at 2000 x *g*.

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

11. 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.

12. Add 5 mL 70% ethanol and vortex for 5 s.

13. Centrifuge for 3 min at 2000 x *g*.

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

14. 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.

15. 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.

16. Add 1 mL 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.

    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.

Protocol: Isolation of DNA from 100–500 µL Buffy Coat

Important points before starting

- The buffer volumes given in the following protocol are suitable for isolation of DNA from 300 µL buffy coat. The protocol can be adapted for DNA isolation from buffy coat samples of 100–500 µL by scaling reagent volumes down or up in proportion to the volume of buffy coat used (see Table 9). However, the volume of Buffer FG3 used to dissolve the DNA in step 13 should remain at 200 µL for all starting volumes of buffy coat.

Table 9. Reagent volumes required for 100–500 µL buffy coat samples

	Buffy coat volume (µL)				
	100	**200**	**300**	**400**	**500**
Buffer FG1	250	500	750	1000	1250
Buffer FG2/QIAGEN Protease	100	200	300	400	500
100% isopropanol	100	200	300	400	500
70% ethanol	100	200	300	400	500
Buffer FG3	200	200	200	200	200

- All centrifugation steps should be carried out at room temperature in a fixed-angle rotor.
- For DNA isolation from buffy coat samples of ≥300 µL to 500 µL, 2 mL centrifuge tubes must be used.

Things to do before starting

- Buffy coat is a leukocyte-enriched fraction of whole blood. Preparing a buffy coat fraction from whole blood is simple and yields approximately 5–10 times more DNA than an equivalent volume of whole blood. Prepare buffy coat by centrifuging whole blood at 2500 x *g* for 10 min at room temperature. After centrifugation, 3 different fractions are

distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat; and the bottom layer contains concentrated erythrocytes.

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit.
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Frozen buffy coat should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Calculate the total volume of buffy coat to be processed. For every 1 mL of buffy coat, mix together 1 mL Buffer FG2 (denaturation buffer) and 10 µL reconstituted QIAGEN Protease (see Table 10). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.

Table 10. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	100	300	500	1000	3000	5000	6000
Volume of Buffer FG2 (µL)	100	300	500	1000	3000	5000	6000
Volume of QIAGEN Protease (µL)	1	3	5	10	30	50	60

Procedure

1. Pipette 750 µL Buffer FG1 into a 1.5 mL centrifuge tube. Add 300 µL buffy coat and mix by inverting the tube 5 times
2. Centrifuge for 20 s at 10,000 x *g* in a fixed-angle rotor
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.

4. Add 300 µL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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 30 µL Buffer FG2 and vortex again.

5. Centrifuge the tube briefly (3–5 s), place it in a heating block or water bath, and incubate at 65°C for 10 min.

6. Add 300 µL 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.

7. Centrifuge for 3 min at 10,000 x *g*.

   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.

   The DNA should be visible as a small white pellet.

9. Add 300 µL 70% ethanol and vortex for 5 s.

10. Centrifuge for 3 min at 10,000 x *g*.

    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.

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.

13. Add 200 µL Buffer FG3, vortex for 5 s at low speed, and dissolve the DNA by incubating for 30 min at 65°C in a heating block or water bath.

    Note: If the DNA is not completely dissolved, prolong the incubation until the DNA is dissolved.

Protocol: Isolation of DNA from 1–2 mL Buffy Coat

Important points before starting

- The buffer volumes given in the following protocol are suitable for isolation of DNA from 2 mL buffy coat samples. The protocol can be adapted for DNA isolation from buffy coat samples of 1–2 mL by scaling reagent volumes down in proportion to the volume of buffy coat used (see Table 11). However, the volume of Buffer FG3 used to dissolve the DNA in step 13 should not be reduced to less than 300 µL.

Table 11. Reagent volumes required for 1–2 mL buffy coat samples

	Buffy coat volume (mL)		
Sample protocol	**1.0**	**1.5**	**2.0**
Buffer FG1	2.5	3.75	5.0
Buffer FG2/QIAGEN Protease	1.0	1.5	2.0
100% isopropanol	1.0	1.5	2.0
70% ethanol	1.0	1.5	2.0
Buffer FG3	300	350	400

- All centrifugation steps should be carried out at room temperature in a swing-out rotor, using conical tubes.

Things to do before starting

- Prepare buffy coat, see page 31.
- Frozen buffy coat should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.
- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):

- 0.3 mL when using 50 mL FlexiGene DNA Kit.
- 1.4 mL when using the 250 mL FlexiGene DNA Kit.
- Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.

- Calculate the total volume of buffy coat to be processed. For every 1 mL of buffy coat, mix together 1 mL Buffer FG2 (denaturation buffer) and 10 µL reconstituted QIAGEN Protease (see Table 12). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.

Table 12. Volumes of Buffer FG2 and QIAGEN Protease required for different batch volumes

Total volume of blood in batch (µL)	1	3	5	10	30	50	60
Volume of Buffer FG2 (µL)	1	3	5	10	30	50	60
Volume of QIAGEN Protease (µL)	10	30	50	100	300	500	600

Procedure

1. Pipette 5 mL Buffer FG1 into a 15 mL centrifuge tube. Add 2 mL buffy coat and mix by inverting the tube 5 times.
2. Centrifuge for 5 min at 2000 x *g* in a swing-out rotor.
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.
4. Add 2 mL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), 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.

5. Invert the tube 3 times, place it in a heating block or water bath, and incubate at 65°C for 10 min.
6. Add 2 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.
7. Centrifuge for 3 min at 2000 x *g*.

   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.

   The DNA should be visible as a small white pellet.
9. Add 2 mL 70% ethanol and vortex for 5 s.
10. Centrifuge for 3 min at 2000 x *g*.

    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.
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.

13. Add 400 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.

    Note: If the DNA is not completely dissolved, prolong the incubation until the DNA is dissolved. If a reduced volume of Buffer FG3 is used, incubation may need to be prolonged.

Protocol: Isolation of DNA from 1–2 x 10^6 Cultured Cells

Important point before starting

- Frozen samples should be thawed quickly in a 37°C water bath with mild agitation and stored on ice before beginning the procedure.

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in the following volumes of Buffer FG3 (hydration buffer):
  - 0.3 mL when using 50 mL FlexiGene DNA Kit.
  - 1.4 mL when using the 250 mL FlexiGene DNA Kit.
  - Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Calculate the number of samples to be processed. For each sample containing 1–2 x 10^6 cultured cells, mix together 300 µL Buffer FG2 (denaturation buffer) and 3 µL reconstituted QIAGEN Protease (see Table 13). The Buffer FG2/QIAGEN Protease mixture should be prepared not more than 1 hour before use.
- Heat a heating block or water bath to 65°C for use in steps 5 and 13.
- All centrifugation steps should be carried out at room temperature in a fixed-angle rotor.

Table 13. Volumes of Buffer FG2 and QIAGEN Protease required for different numbers of samples

Number of samples	1	6	12
Volume of Buffer FG2 (µL)	300	1800	3600
Volume of QIAGEN Protease (µL)	3	18	36

Procedure

1. Harvest cells according to their type of growth:
   Note: Do not use more than 2 x 10^6 cells.

- Cells grown in suspension

  1a. Determine the number of cells.
  1b. Centrifuge the appropriate number of cells for 5 min at 300 x *g* in a 1.5 mL microcentrifuge tube.
  1c. Remove the supernatant completely and discard, taking care not to disturb the pellet.
  1d. Continue with step 2.

- Cells grown in a monolayer
  Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper.

  By *trypsinization*:
  1a. Aspirate the medium and wash cells with PBS.**
  1b. Aspirate the PBS and add 0.10–0.25% trypsin.*
  1c. After the cells have detached from the dish or flask, collect them in medium,* and transfer the appropriate number of cells (maximum 2 x 10^6 cells) to a 1.5 mL microcentrifuge tube.
  1d. Centrifuge for 5 min at 300 x *g*.
  1e. Remove the supernatant completely and discard, taking care not to disturb the pellet. Continue with step 2.

  By using a *cell scrapper*:
  1a. Detach cells from the dish or flask.

* 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.

1b. Transfer the appropriate number of cells (maximum 2 x 10^6 cells) to a 1.5 mL microcentrifuge tube and centrifuge for 5 min at 300 x *g*.

1c. Remove the supernatant completely and discard, taking care not to disturb the pellet.

1d. Continue with step 2.

2. Add 300 µL Buffer FG1 to the cell pellet and mix by pipetting up and down until the cells are resuspended.

   Note: The lysate has a cloudy appearance.

3. Centrifuge for 20 s at 10,000 x *g* in a fixed-angle rotor.

4. Discard the supernatant and leave the tube inverted on a clean piece 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.

5. Add 300 µL Buffer FG2/QIAGEN Protease (see "Things to do before starting"), close the tube and invert 3 times. Place the tube in a heating block or water bath, and incubate at 65°C for 10 min.

6. Add 600 µL 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.

7. Centrifuge for 3 min at 10,000 x *g*.

   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.

   The DNA should be visible as a small white pellet.

9. Add 600 µL 70% ethanol and vortex for 5 s.
10. Centrifuge for 3 min at 10,000 x *g*.

    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.
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.
13. Add 200 µL Buffer FG3, vortex for 5 s at low speed, and dissolve the DNA by incubating for 30 min at 65°C in a heating block or water bath.

    Note: If the DNA is not completely dissolved, prolong the incubation until the DNA is dissolved. With some cell types, traces of the pellet that do not contain DNA may remain undissolved after 1 h incubation at 65°C. If required, they can be removed by centrifuging for 1 min at 10,000 x *g*, and transferring the supernatant into a fresh tube. If a reduced volume of Buffer FG3 is used, incubation time may need to be prolonged.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Low DNA yield		
a)	Protease dissolved in the wrong buffer, or the wrong amount of buffer	Repeat the purification procedure with a new sample, resuspending QIAGEN Protease in the correct volume of Buffer FG3, as described in the protocol.
b)	Buffer FG2/QIAGEN Protease mixture incorrectly prepared or stored	Ensure the mixture of Buffer FG2/QIAGEN Protease is prepared as described in the relevant protocol.
c)	Incomplete sample lysis	Sample was not thoroughly mixed with Buffer FG1. Repeat the purification procedure with a new sample, ensuring that the sample and Buffer FG1 are mixed immediately and thoroughly. Blood sample contained clots. Use only the noncoagulated fraction of the sample as starting material.
d)	Pellet was difficult to resuspend, or was loose and discarded with the supernatant	Centrifugation speed was incorrect. Follow centrifugation recommendations given in the protocol. In rare cases, it may be necessary to optimize centrifugation speed and duration.
e)	Jelly-like traces of pellet remaining after resuspension of pellet in Buffer FG2/QIAGEN Protease	After addition of Buffer FG2/QIAGEN Protease, sample was left for more than 1 minute before vortexing. Incubation times longer than 1 minute must be avoided. If the pellet does not dissolve completely, add the appropriate volume of Buffer FG2 given in the protocol and vortex again.
f)	No DNA precipitate visible after addition of isopropanol	Sample was not thoroughly mixed with isopropanol. Invert sample thoroughly after addition of isopropanol. The DNA should precipitate, and will be visible as threads or a clump. Blood or buffy coat sample had very low white blood cell count, and the DNA pellet may not be visible after centrifugation. Mark the orientation of the tube in the centrifuge, and aspirate the supernatant from the tube wall opposite the pellet after centrifugation. Use a smaller volume of Buffer FG3 to resuspend the pellet. If possible, repeat the DNA isolation procedure with more starting material.

		Comments and suggestions
g)	No DNA pellet visible	Centrifugation speed after addition of isopropanol was too low. Use the centrifugation speed given in the protocols to pellet the DNA. Blood or buffy coat sample had very low white blood cell count. See "No DNA precipitate visible after addition of isopropanol", comment 2, above.
h)	DNA pellet had jelly-like consistency	Mixing with isopropanol was incomplete. Add the same volume of isopropanol as before and vortex until DNA precipitation is complete. Repeat the centrifugation step to pellet the DNA.
i)	DNA pellet lost	Centrifugation speed was too low or centrifugation time was too short. Use the centrifugation conditions given in the protocols. If the DNA pellet becomes detached from the tube wall, centrifuge again or carefully aspirate the supernatant from the tube using a pipette.
j)	DNA was over-dried	Overdried genomic DNA is difficult to dissolve. Prolong the incubation time used to resuspend the DNA to 2 h, then leave the solution on a shaker–incubator at room temperature overnight.
k)	No DNA visible after agarose gel electrophoresis	DNA yield was low due to a low white blood cell count in the starting material. Load the gel with a larger amount of the purified DNA if possible, or repeat the purification procedure with a larger amount of starting material. The DNA can also be dissolved in a smaller volume of Buffer FG3 at the end of the procedure, but the incubation time may need to be prolonged.
Low DNA purity and/or length is short		
a)	Beige DNA pellet	Jelly-like traces of pellet were overlooked after resuspension in Buffer FG2/QIAGEN Protease. Immediate vortexing is important to achieve complete homogenization. Tubes must be left for no more than 1 min before vortexing. Traces of pellet with a jelly-like consistency (barely visible) may remain. If this occurs, add the appropriate volume of Buffer FG2 given in the protocol and vortex again. Despite the appearance of the pellet, it is often worth attempting to use the DNA in downstream PCR. If PCR fails, repeat the procedure with a new sample, ensuring that the pellet is vortexed immediately after addition of Buffer FG2/QIAGEN Protease. Protease digestion was incomplete. Ensure that the QIAGEN Protease is reconstituted and stored according to instructions. Prepare Buffer FG2/QIAGEN Protease mixture not more than 1 h before use.
b)	A_{260}/A_{280} ratio of the purified DNA is less than 1.65	Protease digestion was incomplete. Ensure that the QIAGEN Protease is reconstituted and stored according to the instructions. Prepare Buffer FG2/QIAGEN Protease mixture not more than 1 h before use.
c)	Purified DNA <20 kb in length (measured by agarose gel electrophoresis)	DNA was degraded during storage of the blood sample. If high-molecular-weight DNA is required, store blood no longer than 3–4 days at 2–8°C to avoid DNA degradation by apoptosis. For longer storage, we recommend freezing the samples at –70°C.

	Comments and suggestions	
Purified DNA does not perform well in downstream enzymatic reactions		
a)	Final DNA solution contaminated with ethanol	Buffer FG3 was added to the pellet before all the ethanol had evaporated. Leave the tube inverted on a clean piece of absorbent paper for at least 5 min to minimize backflow of ethanol from the rim and sides of the tube onto the pellet.
b)	Too much DNA used in downstream reaction	PCR is often inhibited by excess DNA. Reduce the amount of DNA used
c)	Too little DNA used in downstream reaction	If the white blood cell count of the starting material was low, the yield of purified DNA may be low. Increase the amount of purified DNA added to the downstream reaction.
d)	Setup of the downstream reaction sub-optimal	Optimize the assay conditions.

Ordering Information

Product	Contents	Cat. no.
FlexiGene DNA Kit (250)	For purification of DNA from 250 mL whole blood: Buffers, QIAGEN Protease	51206
Related products		
QIAamp® DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, Protease, Reagents, Buffers, Collection Tubes (2 mL)	51104
QIAamp DNA Blood Midi Kit (20)*	For 20 DNA midipreps: 20 QIAamp Midi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (15 mL)	51183
QIAamp DNA Blood Maxi Kit (10)*	For 10 DNA maxipreps: 10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 mL)	51192
QIAamp 96 DNA Blood Kit (4)*†	For 4 x 96 DNA preps: 4 QIAamp 96 Plates, QIAGEN Protease, Reagents, Buffers, Lysis Blocks, Tape Pads, Collection Vessels	51161
QIAamp DNA Blood BioRobot MDx Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, S-Blocks, Disposable Troughs, Racks with Elution Microtubes CL (0.4 mL) Top Elute Fluid, Caps, Tape Pad	965152

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** or can be requested from QIAGEN Technical Services or your local distributor.

* Larger kit sizes available; please inquire.

† Requires use of the QIAGEN 96-Well–Plate Centrifugation System.

Document Revision History

Date	Changes
11/2023	Reinstated missing centrifugation step after adding Buffer FG1 to the cell pellet in “Protocol: Isolation of DNA from 1–2 x 10^6 Cultured Cells”. Updated the handbook according to redesigned branding guidelines. Removed FlexiGene DNA Kit (50) and QIAamp DNA Blood BioRobot 9604 Kit (12) from “Ordering Information” table.

Limited License Agreement for FlexiGene DNA Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.