GFX™ Genomic Blood DNA Purification Kit

Data File
Molecular Biology

Description

GFX™ Genomic Blood DNA Purification Kit purifies DNA from whole blood, buffy coat, bone marrow, nucleated red blood cells (RBCs), cultured cells, lymphocytes and buccal cells. With additional reagents, Gram-positive and -negative bacteria and yeast can also be used. DNA purification with GFX relies on a chaotropic agent for cell lysis and the binding of genomic DNA to a glass fiber matrix that is prepacked in a MicroSpin* Column (1, 2). Glass-bound DNA is washed to remove contaminants and the purified DNA is eluted, ready to use, in a low ionic strength buffer (TE, Tris-HCl or water). Purified DNA can be used in a variety of applications including restriction digests, PCR and Southern hybridizations. To efficiently process 24 to 96 samples simultaneously, the kit can be used with either MicroPlex® 24 or MicroPlex 24 Vacuum=.

GFX Genomic Blood DNA Purification Kit provides three purification methods. The Direct Method can process up to 100 µl of whole blood or up to 5 µl of blood containing nucleated RBCs (nucleated blood), and typically yields 2 to 4 µg of DNA from 100 µl of human whole blood and 10 to 15 µg from 5 µl of nucleated blood. The Scalable Method has an additional RBC lysis step and can process up to 1.0 ml of whole blood or bone marrow and up to 50 µl of buffy coat. Typical yields with the Scalable Method are 4.5 to 7.5 µg of DNA from 300 µl of human whole blood. The Cultured Cells and Lymphocyte procedure can process up to 5 x 10^6 cultured cells or up to 1 x 10^7 lymphocytes; average recoveries range from 8 to 14 µg for 2 x 10^6 cultured cells.

GFX Genomic Blood DNA Purification Kit offers:

- **Speed and ease.** Isolate DNA using the Direct Method or the procedure for Cultured Cells and Lymphocytes in just 15 minutes. The Scalable Method can be completed in 20 minutes. GFX methods eliminate dispensing glass slurries, batch washings and ethanol precipitations.
- **Consistent yield and purity.** The Direct Method typically yields 2 to 4 µg from 100 µl of human whole blood. The Scalable Method yields 4.5 to 7.5 µg from 300 µl of human whole blood with an average A_{260}/A_{280} of 1.7.

= U.S. Patent No. 5,603,899 has been issued to Amersham Biosciences Inc. for multiple column chromatography assembly.

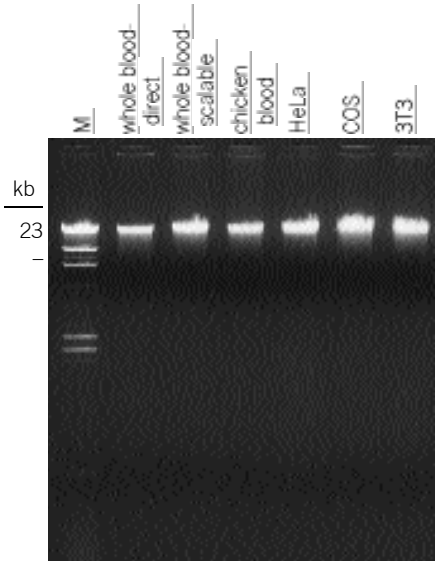

Figure 1. Genomic DNA isolated from various sources using GFX™ Genomic Blood DNA Purification Kit. For each sample, DNA was eluted using 200 µl of TE buffer (pH 8.0). Each lane contains 250 ng of DNA as determined by absorbance at 260 nm. Whole blood-direct = 100 µl of human whole blood processed using the Direct Method; whole blood scalable = 300 µl of human whole blood processed using the Scalable Method; chicken blood = 2.5 µl of chicken blood processed using the Direct Method; HeLa = 1 x 10^6 cells; COS = 2 x 10^6 cells; 3T3 = 2 x 10^6 cells. Cultured cells were processed using the procedure for Cultured Cells and Lymphocytes. M = λ DNA-*Hin*d III Digest (27-4048-01).

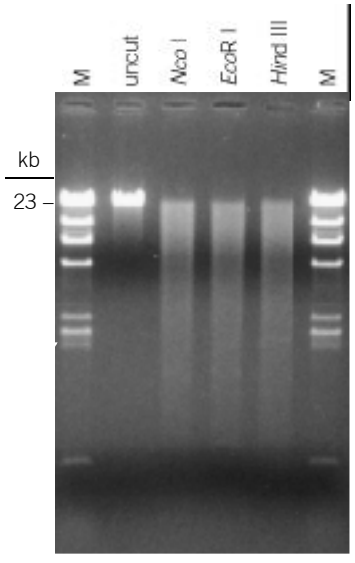

Figure 2. Restriction endonuclease digests of human genomic DNA isolated from 300 µl of whole blood using GFX™ Genomic Blood DNA Purification Kit with the Scalable Method. DNA was digested using 0.5 µg of genomic DNA and 5 units of enzyme in a total volume of 30 µl 1X One-Phor-All Buffer *PLUS*. Samples were incubated at 37°C for 2 hours. Aliquots (15 µl) were analyzed by agarose gel electrophoresis. M = λ DNA-*Hin*d III Digest (27-4048-01).

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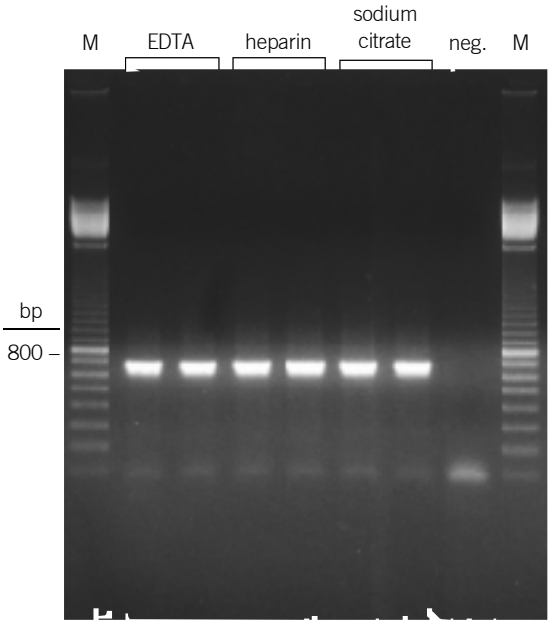

Figure 3. Duplicate 300 µl samples of rabbit blood stored in different anti-coagulants (EDTA, heparin or sodium citrate) were processed using GFX™ Genomic Blood DNA Purification Kit with the Scalable Method. Purified genomic DNA was eluted using 200 µl of TE buffer (pH 8.0). 10 µl of each purified sample was used for PCR with primers specific for rabbit β-globin. Reactions were subjected to 35 cycles of 94 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute. One-tenth of each reaction was loaded onto a lane of an agarose gel. neg. = no template control PCR. M = 100 Base-Pair Ladder (27-4001-01).

- **Robustness.** Isolate DNA from a variety of samples, including whole blood, nucleated blood, bone marrow, buffy coat, cultured cells, lymphocytes, buccal cells, bacteria and yeast.
- **Multiple sample handling.** GFX Genomic Blood DNA Purification Kit can be used with either MicroPlex 24 or MicroPlex 24 Vacuum (each purchased separately) to process 24 to 96 samples simultaneously**.
- **No toxic reagents.** No hazardous reagents such as phenol or chloroform are required.

Components

GFX Genomic Blood DNA Purification Kit provides sufficient material to perform 100 purifications, each using 300 µl of whole blood.

GFX Genomic Blood DNA Purification Kit includes the following components:

RBC Lysis Solution: 10 mM $KHCO_3$, 155 mM NH_4Cl, 0.1 mM EDTA (3).

Extraction Solution: Buffered solution containing chaotrope and detergent.

** For information on processing samples with MicroPlex 24 Vacuum, contact your Amersham Biosciences representative.

Wash Solution: Tris-EDTA buffer with absolute ethanol (ethanol not supplied).

GFX Columns: MicroSpin* columns pre-packed with a glass fiber matrix.

Collection Tubes: Capless 2 ml microcentrifuge tubes.

Quality Control

Each lot of GFX Genomic Blood DNA Purification Kit is tested for the ability to isolate genomic DNA of high yield and quality from human blood.

Shipping and Storage

Ship: Ambient.

Store: Ambient.

References

1. Vogelstein, B. and Gillespie, D., *Proc. Natl. Acad. Sci. USA* **76,** 615 (1979).
2. Marko, M. A. *et al.*, *Anal. Biochem.* **121,** 382 (1982).
3. Rolfs, A., Schuller, I., Finckh, U. and Weber-Rolfs, I., *PCR:Clinical Diagnostics and Research*. Springer-Verlag (1992).

Ordering Information

Code No.	Pack Size
GFX™ Genomic Blood DNA Purification Kit	
27-9603-01	100 purifications
Companion Products	
MicroPlex® 24	
27-3564-01	1 set
MicroPlex® 24 Vacuum	
27-3567-01	1 system
Ready-To-Go® PCR Beads (0.5 ml tubes)	
27-9555-01	100 reactions
Ready-To-Go® PCR Beads (0.2 ml tubes/plate)	
27-9553-01	96 reactions/plate
GFX™ PCR DNA and Gel Band Purification Kit	
27-9602-01	100 purifications

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