Application Note:
AN-LECF-GENDNA-0308

Isolating Genomic DNA from Whole Blood

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KEY WORDS

- Centrifugation
- Genomic DNA Isolation
- Nucleon BACC2 Kit

Abstract

The collection of pure high molecular weight genomic DNA from whole blood or cell cultures is a key starting step for researchers conducting downstream research such as PCR amplification or restriction digests. In this protocol, high purity DNA was collected from the buffy coat layer of human blood from 10 patients for downstream analysis. Using the Nucleon® BACC2 kit, DNA results were analyzed to prove acceptable yield and purity in the Thermo Scientific refrigerated microcentrifuge with 24-place 1.5/2.0 ml rotor.

Readily available kits, such as the Nucleon BACC2 from GE® Health Care Europe-Amersham, minimize the need for reagents and consumables. The end-user only has to provide a high quality refrigerated microcentrifuge and pipettes to complete the isolation process. It is important to find a suitable DNA isolation system and to use appropriate equipment to satisfy downstream applications, including enzyme applications and cDNA synthesis.

The Thermo Scientific 24-place refrigerated microcentrifuge proved to be ideal for use with DNA extraction kits such as the Nucleon BACC2 kit.

Figure 1. Thermo Scientific refrigerated microcentrifuge with 24-place rotor.

Materials and Methods

Human blood samples were collected from 10 patients. DNA was extracted from the buffy coat layer, following the instructions included with the Nucleon BACC2 kit from GE Health Care Europe-Amersham. The protocol described in this application note is a summary of the procedure included in the kit manual provided by the manufacturer. Refer to the kit manual for detailed product information and protocols.

All centrifugation steps were performed using the Thermo Scientific refrigerated micro-centrifuge with the standard 24-place 1.5/2.0 ml rotor at 4°C.

Procedure

Cell lysis

1. In a 1.5 ml microcentrifuge tube, resuspend 400 µl of the buffy coat in 1 ml of solution A (sodium perchlorate).
2. Pellet the cells in the refrigerated microcentrifuge's 24-place rotor by centrifuging at 6000 rpm for 5 minutes at 4°C.
3. Discard the supernatant and resuspend the cell pellet 2 or 3 times in 700 µl of solution A (sodium perchlorate).

Deproteinization with sodium perchlorate

4. Discard the supernatant and resuspend the cell pellet in 500 µl of reagent B (chloroform) with brief vortexing.
5. Add 125 µl of sodium perchlorate and invert the tube several times.

Extraction with chloroform and Nucleon resin

6. Add 500 µl of chloroform and invert the tube for 20-30 seconds.
7. Add 150 µl of Nucleon silica and centrifuge at 6500 rpm in the refrigerated microcentrifuge for 3 minutes at 4°C.

DNA precipitation

8. Transfer the upper phase (500 µl) to a fresh 1.5 ml tube and precipitate the DNA with 2 volumes of cold absolute ethanol.
9. Centrifuge at 12,000 rpm in the Thermo Scientific refrigerated microcentrifuge for 10 minutes at 4°C.

DNA washing

10. Wash the DNA with 1 ml of 70% cold ethanol.
11. Pellet the DNA again, centrifuging at 12,000 rpm in the Thermo Scientific refrigerated microcentrifuge for 10 minutes.
12. Air dry and resuspend the DNA in TE buffer.

Evaluation of nucleic acid purity
Optical density measurements were used to assay the DNA yield and check for contamination by salts, solvents and proteins. To evaluate the purity of the extracted DNA, absorbance ratios at 260 nm/230 nm (DNA/Organic or carbohydrates contaminants like phenol and other aromatic compounds) and 260 nm/280 nm (DNA/protein) were determined. (A typical yield is 200-400 µg of DNA with a 260/280 ratio of 1.8 to 2.)

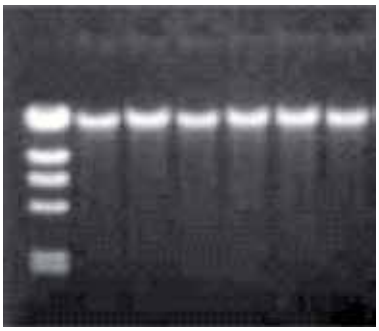

Figure 2. DNA isolated from human blood following the Nucleon protocol kit and using the Thermo Scientific refrigerated microcentrifuge. DNA was separated on a 1 % agarose gel. Lane 1 contains I x Hind III molecular weight markers, lanes 2-7 contain samples.

Results

Evaluation of DNA purity
Optical density measurements were taken after DNA extraction using the Nucleon BACC2 kit and the Thermo Scientific refrigerated microcentrifuge. An OD 260/280 ratio of 1.8 to 2 and an OD 260/230 ratio of 1.8 or greater indicate that good quality DNA was obtained (see Table 1, Figure 2). Pure DNA is then ready for downstream processes.

Conclusion

High yield, high purity DNA was obtained from human blood using the Nucleon BACC kit system with the Thermo Scientific refrigerated microcentrifuge and 24-place 1.5/2.0 ml rotor. DNA prepared in this way is ready to be digested with restriction endonucleases and has been employed for DNA profiling and real-time PCR.

The user-friendly Thermo Scientific refrigerated microcentrifuge with 24-place 1.5/2.0 ml rotor proved to be an outstanding performer for constant stable refrigeration and is recommended for all laboratories using standard methods or kits for isolation of genomic DNA or other kit protocols.

Sample ID	Quantity ng/µl	OD 260/280	OD 260/230
Patient 1	476.71	1.87	2.11
Patient 2	534.07	1.88	1.96
Patient 3	546.04	1.88	1.83
Patient 4	791.17	1.92	1.84
Patient 5	805.83	1.91	1.90
Patient 6	611.87	1.89	1.80
Patient 7	695.31	1.90	1.83
Patient 8	533.25	1.87	1.88
Patient 9	562.41	1.87	1.84
Patient 10	648.24	1.90	1.83

Table 1. Optical density measurements achieved using the Nucleon BACC2 kit and the Thermo Scientific refrigerated microcentrifuge with 24-place 1.5/2.0 ml rotor.

References

GE Health Care Europe-Amersham; Product Codes RPN 8501, 8502 or 8512; Nucleon BACC, Genomic DNA from Blood and Cell Cultures publication number 18-1146-65, Rev 1.

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