Application Note:
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DNA Preparation from Large Volume of Whole Blood Using the New Thermo Scientific General Purpose Centrifuge with Fiberlite Rotors

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KEY WORDS

- Whole Blood
- DNA Purification
- Phenol/Chloroform Method
- General Purpose Centrifuge
- Fiberlite High Capacity Rotors

Introduction

In the fields of pharmacogenetics and clinical diagnostics, there is a major increase in the demand for high quality DNA and RNA. In these fields, the most common source of DNA is whole blood (see Figure 1). The blood is collected, typically in larger volumes (10 mL).

DNA samples are purified using the standard manual phenol/chloroform method, which uses human whole blood samples collected from a single donor.

The following application brief describes the preparation of genomic DNA (gDNA) using the step-run feature of the new Thermo Scientific general purpose centrifuge series with a Thermo Scientific Fiberlite F13-14x50c (or F15-8x50c) carbon fiber conical tube rotor. These solutions provide the high capacity required for laboratories performing large-sample nucleic acid purification.

Thermo Scientific General Purpose Centrifuge with Fiberlite F15-8x50c Rotor

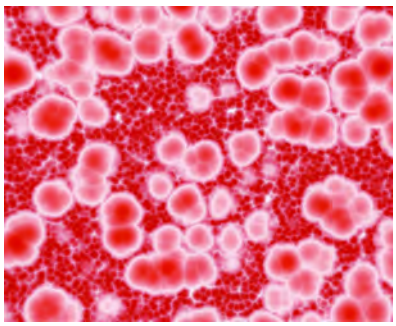

Figure 1: Blood cells

Procedure

Isolation of gDNA from Whole Blood

The phenol/chloroform method[1] outlined in this brief provides a step-by-step procedure for isolation of gDNA from whole blood using 50 mL disposable conical tubes in a Fiberlite® F13-14x50c rotor and a new Thermo Scientific general purpose centrifuge.

1. To 10 mL whole blood (EDTA, heparin, citrate), add 30 mL lysis buffer. Shake gently, incubate for 30 min on ice, then centrifuge at 1200 rpm for 10 min at 4°C.
2. Remove supernatant (blood waste). Add 10 mL lysis buffer, resuspend the pellet, and spin at 1200 rpm for 10 min at 4°C.
3. Remove supernatant (blood waste). Add 5 mL SE-buffer, resuspend the pellet, and centrifuge at 1200 rpm for 10 min at 4°C.
4. Remove supernatant (blood waste). Add 5 mL SE-buffer and resuspend the pellet. Add 40 µL proteinase K (10 mg/mL) and 250 µL 20% SDS. Shake gently and incubate 1 h at 55°C in a water bath.
5. Add 5 mL SE-buffer and 10 mL phenol. To remove proteins from nucleic acid solution, shake by hand and centrifuge at 3000 rpm for 5 min at room temperature.
6. Recover and transfer the aqueous (top) phase containing the nucleic acid into a clean tube. Add 10 mL phenol/chloroform/isoamyl alcohol (25:24:1). Shake by hand and centrifuge at 3000 rpm for 5 min at room temperature.

Thermo Scientific Fiberlite F13-14 x 50c Rotor