

AutoGenprep 965 Application Guide

Buffy Coat



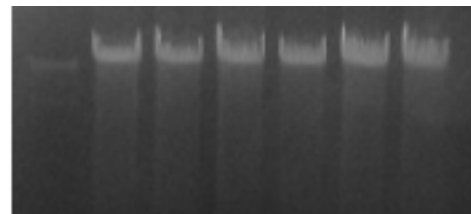
- I. **Purpose:** To extract DNA from EDTA or acid-citrate-dextrose treated buffy coat.
- II. **Chemical Principal:** Modified GTC (Guanidine thiocyanate) extraction chemistry
- III. **Protocol Parameters:**
 - A. **Sample Volume:** 100-200ul Buffy coat derived from up to 2ml whole blood (fresh or frozen).
 - B. **Maximum Number of Samples:** Up to 384 (4 of 96 plates) samples per run.
 - C. **Processing Time:** 4.2 hours for 192 samples
6.4 hours for 384 samples.
This includes a 60 minute DNA drying time
 - D. **Yield:** ~25 µg of purified gDNA / 100µl of buffy coat derived from 2ml whole blood.
 - E. **Quality:** Typical OD260/280 values are 1.7 ~ 1.9
The DNA can be used in downstream processes such as PCR, real time PCR, Microarrays.
- IV. **Running the Protocol:**
 - A. Load Reagents and Samples
 - B. Select a Protocol
 - C. Enter Number of Samples
 - D. Start the Run

V. Example of genomic DNA obtained from buffy coat:

5ul Genomic DNA loaded in lanes 2-7.
48.5kb ladder in lane 1

Starting material: 100µl of EDTA treated buffy coat
derived from 2ml frozen whole blood.

Gel condition: 0.7% agarose gel, 1xTBE, 80V x 1hr



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated	Lyse white blood cells	Add Reagent WB-R1* and mix.
2. Automated	Denature protein	Add Reagent WB-R2* and mix.
3. Automated	Precipitate protein	Add Reagent WB-R3* and mix.
4. Automated	Collect DNA	Add Reagent WB-R4*, mix, centrifuge and transfer supernatant to a new plate.
5. Automated	Precipitate DNA	Add Reagent WB-R5*, mix, centrifuge and discard supernatant.
6. Automated	Wash DNA	Add Reagent WB-R6/7/8*, mix, centrifuge and discard supernatant. Repeat 2 times.
7. Automated	Dry DNA	Evaporate residual solvent.
8. Automated	Resuspend DNA	Add Reagent WB-R9*, mix and centrifuge.

* All reagents and consumables are sold as a complete kit for whole blood DNA extraction for the AutoGenprep 965.

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