

AutoGenFlex STAR Application Guide

Fresh Whole Blood



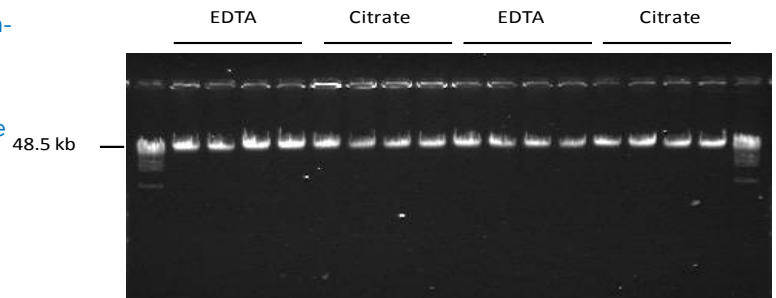
- I. **Purpose:** To extract genomic DNA from EDTA, or citrate treated fresh human whole blood.
- II. **Chemical Principal:** QIAGEN's FlexiGene Procedure (protease digestion/DNA precipitation method)
- III. **Protocol Parameters:**
 - A. **Sample Volume:** 1.0 – 5.0 ml of fresh whole blood or fresh buffy coat prepared from 1.0 – 5.0 ml of fresh whole blood.
 - B. **Maximum Number of Samples:** Up to 40 samples per run.
 - C. **Throughput:** Up to 80 x 5.0 ml or 40 x 10.0 ml samples per day.
 - D. **Yield:** 100 – 210 µg of purified DNA per 5.0 ml of fresh whole blood
 - E. **Quality:** Typical OD260/280 values are 1.70 – 1.90. The DNA is free of contaminants and inhibitors (RNA, protein) and can be used directly in downstream processes such as DNA sequencing, PCR-based assays, restriction digestions, and Southern blotting.

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 fresh whole blood:

Genomic DNA was isolated on the AutoGenFlex using the FlexiGene 5ml fresh whole blood protocol. The starting material was 5.0 ml of EDTA or citrate treated fresh whole blood from four different donors with four replicates each. The yield from 5.0ml whole blood is 100-210 µg genomic DNA with a high molecular weight of 30-150 kb.



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated	Obtain nuclei pellet (includes mitochondria)	Add Buffer FG1*, centrifuge and discard supernatant.
2. Automated	Wash nuclei pellet	Add Buffer FG3*, centrifuge and discard supernatant. Repeat.
3. Automated	Digest/lyse nuclei	Add QIAGEN Protease and Buffer FG2*. Mix, incubate and mix again.
4. Automated	Precipitate DNA	Add R4** or Isopropanol to DNA recovery tube. Transfer supernatant of sample tube into DNA recovery tube, mix, centrifuge and discard the supernatant.
5. Automated	Wash DNA	Add R5** or 70% Ethanol, mix, centrifuge and discard the supernatant.
6. Automated	Resuspend DNA***	Add reagent FG3*, mix, incubate, mix and centrifuge.

* Buffers FG1, FG2 FG3 and QIAGEN Protease are available from AutoGen as AGFSTAR FlexiGene Reagent Kit (640) Catalog #AGKT-FG-640.

** Reagents R4 and R5 are available from AutoGen, Inc. as AutoGenFlex Blood DNA Finishing Kit, Catalog #AGFXPW.

*** Samples can be vortexed after initial incubation if required.

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