Automated Cell Free DNA Extraction using *ExiPrep*™96 Lite from Serum, Plasma and Urine Samples: Simple, Easy and Fast

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Abstract

Bioneer has developed a high-throughput DNA extraction system, *ExiPrep*™96 Lite. We also developed *MagListo*™ cfDNA Extraction Kit for manual extraction. In order to provide automated, high-throughput clinical application of cfDNA extraction, we developed the protocol of *ExiPrep*™96 Lite with *MagListo*™ cfDNA Extraction Kit. It provides automatic cfDNA extraction of 24 samples per run with high quality equal to QIAamp Circulating Nucleic Acid Kit. It can extract cfDNA from a maximum of 4 ml of each sample within 40 minutes. In conclusion, *MagListo*™ cfDNA Extraction Kit is applicable on *ExiPrep*™96 for high-throughput cfDNA purification.

Introduction

Circulating cell-free DNA (cfDNA) are cleaved DNA fragments released to the blood plasma. Recently, cfDNA is a crucial clinical specimen for liquid biopsy not only for early diagnosis of cancer but also for the decision of drug treatment. However, the concentration of cfDNA is generally low, so a large volume of the sample more than 1 ml is required for precise analysis. There are only a few instruments the can handle volume more than 2 ml, and currently available manual kits have complex workflow and long handling time, which would limit large-scale clinical application. In this study, we developed an automated cfDNA extraction protocol with manual kit to solve the limitation previously mentioned.

Materials & Methods

We extracted cfDNA from serum, plasma, and urine from a healthy human. The volume of samples was varying from 1 ml to 4 ml to evaluate performance by volume. We optimized the protocol of *ExiPrep*™96 Lite with A magnetic bead-based cfDNA extraction kit, the *MagListo*™ cfDNA Extraction Kit, to extract high-purity cfDNA efficiently. (Fig.1). For pre-treatment, proper amount of lysis buffer and proteinase K was mixed with the samples and incubated at 60℃ for 10 min. During incubation, a magnetic bead, washing buffer, and elution buffer were dispensed into cartridge 2-7. Then Sample-buffer mixture was moved into the cartridge 1, and the proper amount of isopropanol was added. After loading all buffer cartridges on *ExiPrep*™96 Lite, protocol for cfDNA extraction was selected to run. After running the machine, eluted DNA samples in cartridge 7 were moved into new 1.5 ml tubes. For the comparison, we also manually extract cfDNA with QIAamp Circulating Nucleic Acid kit according to the manufacturer's manual using vacuum manifold, QIAvac 24 Plus. Extracted cfDNA was quantified and visualized using Agilent 5200 Fragment Analyzer System.

Figure 1. *MagListo*™ cfDNA Extraction Kit and *ExiPrep*™96 Lite.

ExiPrep™96 Lite Work Flow

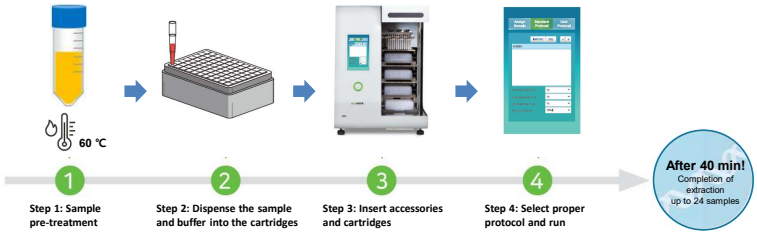


Figure 2. *ExiPrep*™96 Lite cfDNA extraction work flow.

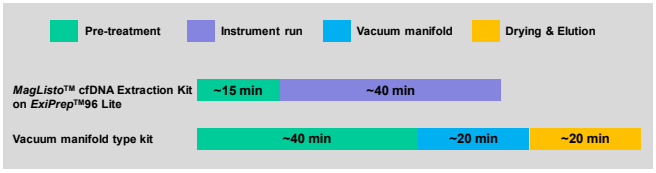


Figure 3. Time schedule of cfDNA extraction using *ExiPrep*™96 Lite and vacuum manifold type kit.

Results

1. cfDNA extraction from plasma, serum and urine

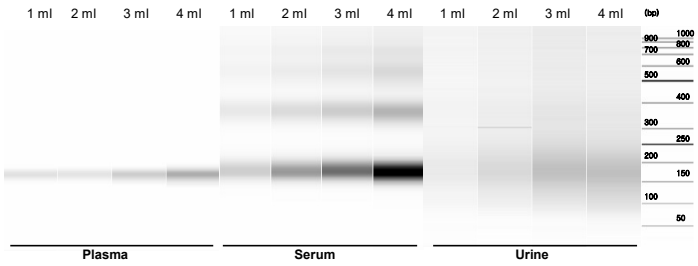


Figure 4. Cell-free DNA was isolated from various volume of plasma, serum and urine using *MagListo*™ cfDNA Extraction Kit on *ExiPrep*™96 Lite. DNA was visualized using Agilent 5200 Fragment Analyzer System. cfDNA yield increases proportionally to the sample volume of plasma, serum and urine. cfDNA concentration of plasma and urine is relative lower than that of serum because old plasma and normal human urine was used for the experiment.

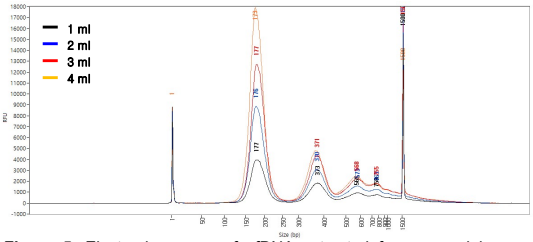
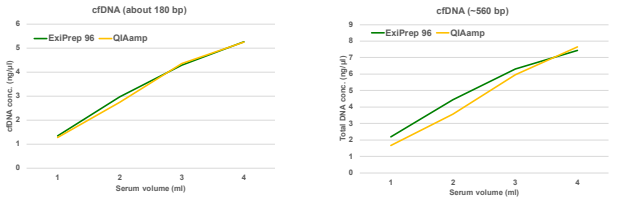


Figure 5. Electropherogram of cfDNA extracted from normal human sample show that Increasing serum volume yield increasing amounts of cfDNA. No genomic DNA was seen. The peaks at the beginning and end are lower and upper marker

2. Performance comparison to vacuum manifold type kit



	Serum volume	Elution	~180 bp Conc. (ng/µl)	~370 bp Conc. (ng/µl)	~560 bp Conc. (ng/µl)	Total Conc. (ng/µl)
ExiPrep™96	1 ml	100 µl	1.3442	0.5707	0.2822	2.1971
	2 ml	100 µl	2.9680	0.9835	0.4970	4.4485
	3 ml	100 µl	4.1951	1.3961	0.7216	6.3128
	4 ml	100 µl	5.2670	1.4068	0.7625	7.4363
QIAamp	1 ml	100 µl	1.2751	0.2552	0.1280	1.6583
	2 ml	100 µl	2.7393	0.5538	0.2731	3.5662
	3 ml	100 µl	4.3613	1.0421	0.5473	5.9507
	4 ml	100 µl	5.2476	1.4905	0.9161	7.6542

Table 1. *MagListo*™ cfDNA Extraction Kit on *ExiPrep*™96 Lite recovers similar concentration of cfDNA to a standard vacuum manifold type kit, QIAamp circulating Nucleic Acid Kit. The DNA concentration increases linearly proportional to serum sample volume. Concentration of the one (~180 bp), two (~370 bp) and three (~560 bp) nucleosomal cfDNA were quantified using Agilent 5200 Fragment Analyzer System.

Conclusions

- *ExiPrep*™96 Lite can handle a maximum of 4 ml volume of 24 samples at once.
- *ExiPrep*™96 Lite with *MagListo*™ cfDNA Extraction Kit can extract similar amount of manual extraction with QIAamp Circulating Nucleic Acid Extraction Kit.
- Operation of *ExiPrep*™96 Lite is straightforward and running time is 40 min enough to finish all the extraction within 1 hr.
- As a result, *ExiPrep*™96 Lite will become a powerful tool for cfDNA clinical application.

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