Automated circulating cell-free DNA purification with the chemagic 360 instrument.

Abstract

The purification of circulating cell-free DNA (cfDNA) is not trivial because of the low concentrations, its instability and the small fragment sizes. Nevertheless the information it delivers is highly valuable for cancer research and research of non-invasive prenatal testing using technologies as NGS and ddPCR. The chemagic™ cfDNA kits enable the automated and efficient extraction of cfDNA from 0.5 - 5 mL plasma in 24-well or 96-well format in maximal 2 hours with minimal hands-on time. The chemagic cfDNA kits show equivalent yields as well as purities compared to the manual silica based gold standard kit.

Introduction

cfDNA is released into the blood plasma upon necrosis and apoptosis from blood and tissue cells, during this process DNA is degraded into nucleosomal units[1]. The research of the nucleosomal fragments showed the potential of cfDNA for monitoring specific mutations to predict pathologic conditions including cancer[2]. The discovery of fetal cfDNA in the maternal circulation[3] has led to multiple studies to learn more about abnormalities in the structure or the number of chromosomes in addition to the fetal sex and rhesus factor. Therefore, the analysis of cfDNA in blood has become an elegant non-invasive method to study cancer or chromosomal disorders.

The isolation of cfDNA represents a huge challenge due to low concentrations (1 - 50 ng/mL plasma), instability and small fragment sizes of about 170 bp (50 - 200 bp) and thus causes a high demand for precise cfDNA purification workflows.

For research use only. Not for use in diagnostic procedures.

Product overview

Table 1: The developed kits cover medium- and high-throughput formats.

Product no.	Kit name	chemagic Rod Head	Format	Preps/ Kits	Sample volumes	Yield
CMG-1304	chemagic cfDNA 5k Kit H24	24	24-well	240	3 - 5 mL	1 - 30 ng/mL plasma (qPCR)
CMG-1302	chemagic cfDNA 2k Kit H24	24	24-well	240	1 - 2 mL	1 - 30 ng/mL plasma (qPCR)
CMG-1396	chemagic cfDNA 1.5k Kit H96	96	96-well	960	0.5 - 1.5 mL	1 - 30 ng/mL plasma (qPCR)

Revvity developed three chemagic™ cfDNA kits to isolate cfDNA from 0.5 - 5 mL fresh and frozen plasma derived from EDTA, citrate or Streck® Cellfree DNA BCT® tubes with the chemagic 360 instrument (Figure 1).

The chemagic cfDNA kits are based on chemagic technology using M-PVA Magnetic Beads for the isolation of cfDNA. The cfDNA binds to paramagnetic beads, which are magnetically separated from the sample material. During subsequent steps, contaminants are removed and the purified cfDNA is transferred into an elution medium. The automated sample processing by the chemagic 360 instrument excludes cross-contamination and ensures safe handling of infectious sample material.

Figure 1: chemagic 360 instrument used for cfDNA isolation with chemagic cfDNA kits.

Results

Nucleic acids were extracted from 1 - 5 mL plasma with the chemagic cfDNA kits CMG-1396, CMG-1302 and CMG-1304 and compared to a manual, silica spin-based competitor extraction kit. Figure 2 shows that a mononucleosomal peak of cfDNA was detected at ~170 bp after extraction with all kits. A dinucleosomal cfDNA peak at ~340 bp was visible when 5 mL plasma was employed in CMG-1304 and competitor. In all cases, the yields achieved by the chemagic cfDNA extraction kits are comparable to the corresponding competitor extraction from 1 - 5 mL plasma. No extraction of high molecular weight DNA from possibly lysed white blood cells was observed with any of the extractions.

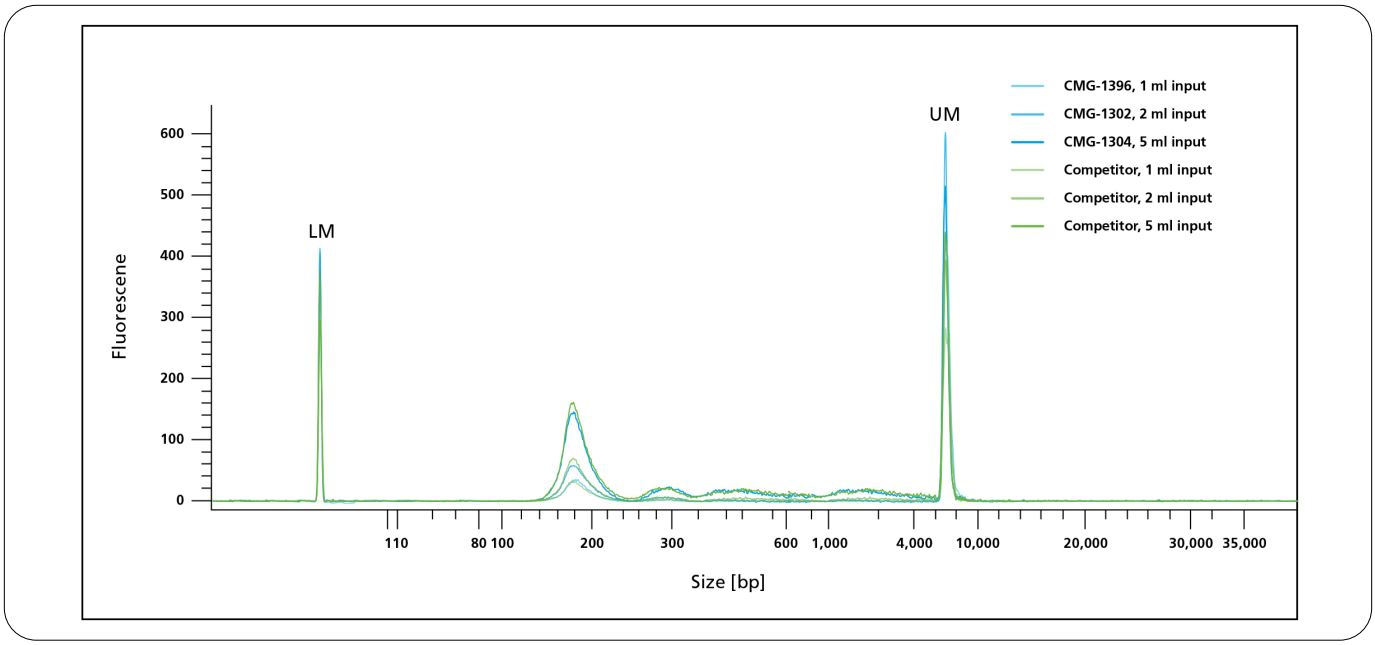

Figure 2: Comparable yields of cfDNA from 1 - 5 mL plasma with chemagic cfDNA kits compared to competitor kits. Plasma was prepared from fresh blood of a healthy donor by centrifugation at 2000 x *g* for 20 minutes. The supernatant was collected and centrifuged once more at 3300 x *g* for 30 minutes. The plasma was frozen at -20 °C and thawed at 30 °C prior to cfDNA extraction. cfDNA was extracted from 1, 2, and 5 mL plasma with the chemagic cfDNA kits on the chemagic 360 instrument (blue lanes) and manually with competitor (green lanes). The eluted DNA was loaded on the HT X-Mark LabChip running the HT DNA NGS 3K assay on the LabChip GX Touch HT Nucleic Acid Analyzer (LM: Lower Marker, UM: Upper Marker).

The scalability of all chemagic cfDNA isolation kits in comparison to a competitor kit was shown using ALU115 qPCR (Figure 3).

A degradation score for the extracted cfDNA was determined by differential qPCR quantitation using the KAPA™ Human Genomic DNA Quantification and QC Kit from Roche as quality control for cfDNA. The kit includes three different primer mixes to amplify 41 bp, 129 bp and 305 bp fragments of a conserved, single-copy human locus. Longer amplicons indicate the presence of high genomic DNA (hgDNA). Thus, if the ratios (degradation scores) of the amplicon concentrations of the 129 bp and 305 bp assay compared to the 41 bp assay (Q129/Q41 and Q305/Q41) are smaller than 1, an increased amount of fragmented DNA, such as cfDNA, is present compared to larger hgDNA. Purification of hgDNA would yield a degradation score of 1. With the new chemagic cfDNA extraction kits, degradation scores in the range of (0.16 - 0.37) for both, Q129/Q41 and Q305/Q4, were obtained. This is comparable to the results gained with the silica membrane based competitor kit on the same samples (degradation scores 0.15 - 0.4).

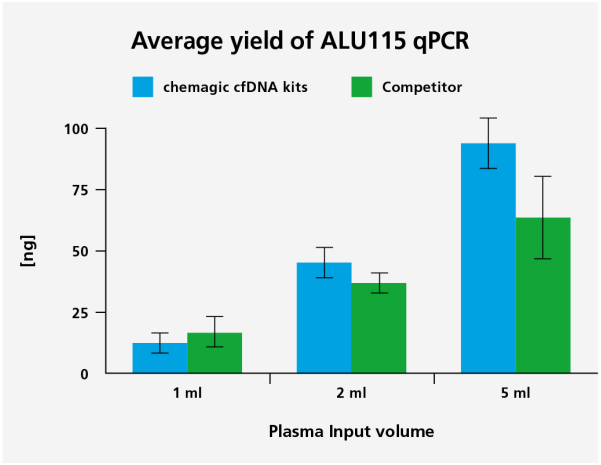

Figure 3: Increasing cf DNA yields with increased sample input. cf DNA was isolated from 1 mL, 2 mL and 5 mL from two different donors both with the chemagic kits on the chemagic 360 instrument and manually with competitor. For cf DNA analysis, a short fragment (115 bp) from a consensus sequence with abundant genomic ALU repeats was amplified[4]. The exemplary data from donor 2 shows that the yield of cf DNA is scalable to sample input.

Summary

The importance of cfDNA increases as it is becoming an essential tool for research of prenatal disorders and malignant diseases. The demand on efficient cfDNA extraction methods from plasma and serum grows accordingly and due to the higher number of samples an automated extraction method would be an advantage.

In this technical note it is shown that the new chemagic cfDNA kits used on the chemagic 360 instrument obtain similar yields compared to a manual competitor silica membrane based kit. The chemagic kits provide a reliable isolation of high yields of cfDNA from 1 - 5 mL plasma per sample. Independent from sample input and throughput, cfDNA was successfully isolated and showed equal and scalable cfDNA yields compared to the competitor kit. In addition, the isolated nucleic acid samples obtained degradation scores smaller than 1 similar to the competitor kit indicating an increased amount of small DNA fragments such as cfDNA.

Literature

1. van der Vaart, M. et al. (2077), The Origin of Circulating Free DNA, Clinical Chemistry; 53 (12): 2215, https://doi.org/10.1373/clinchem.2007.092734
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