DIACARTA®

QuantiDNA™ cfDNA Extraction Kits

Manual kits and automated kits for research use

cfDNA Extraction and Applications

cfDNA (cell-free DNA) has been used as a biomarker for cancer diagnostics and other diseases. Plasma cfDNA is especially widely used compared with other bioliquids such as urine. However, since cfDNA in the blood is mixed with red and white blood cells, extraction of cfDNA is necessary to identify somatic mutations or gene methylation patterns from tumor cells.

Extraction of cfDNA from blood includes two steps: plasma isolation and cfDNA extraction from plasma. We provide manual and automated cfDNA extraction kits (1 to 10 mL plasma per sample).

Manual cfDNA Extraction at a Glance

Here is the workflow for manual cfDNA extraction. After plasma isolation, the two-step extraction by magnetic beads and column is applied to ensure the cfDNA extracted is pure and free of inhibitors on downstream PCR reactions.

Plasma isolation	Proteinase K Treatment	First step extraction	Second step extraction
20 mins	15 mins	5 mins	20 mins

Automated cfDNA Extraction at a Glance

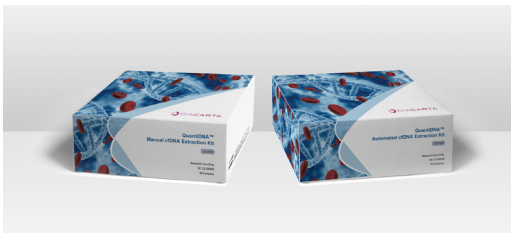
Unlike the manual cfDNA extraction kits, magnetic beads are also used for the second step of cfDNA extraction. The first- and second-step extractions are conducted on the automated extraction instrument MGISP-NE384.

Plasma isolation	Proteinase K Treatment	First step extraction	Second step extraction
20 mins	15 mins	23 mins	41 mins

Product Performance Compared with Qiagen's QIAamp MinElute ccfDNA Midi Kit

Yield, Concentration, and Integrity

cfDNA from 12 healthy donors' plasma (4 mL plasma each) were extracted by QuantiDNA™ kits and QIAamp™ kits in this pilot study. The average concentration of cfDNA isolated using QuantiDNA™–Automated, QuantiDNA™–Manual and QIAamp was 0.37, 0.28, and 0.26ng/μL, respectively. QuantiDNA™ kits could get equal or even more concentrated cfDNA than QIAamp (Fig 1 and 2). After the process of the automated step, the recovered volume of QuantiDNA was around 45 μL, rather than the 60 μL start volume. The average yield of cfDNA among these three methods was 16.8, 17.0, and 15.6ng from 4mL plasma. Bioanalyzer electropherograms show the same typical size distribution for cfDNA extracted by QuantiDNA™ kits and QIAamp kits. No other DNA peaks are found in the cfDNA preparations (Fig 3).



DIACARTA INC. 4385 HOPYARD RD, SUITE 100, PLEASANTON, CA 94588, USA PHONE: +1 800-246-8878 EMAIL: INFORMATION@DIACARTA.COM

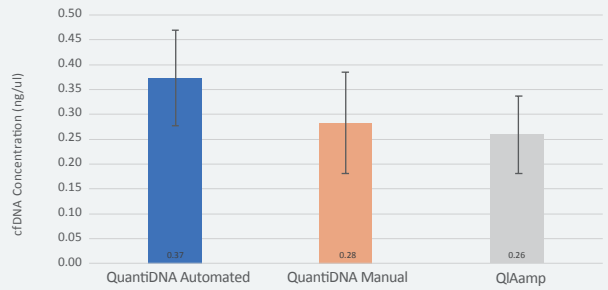


Figure 1. Plotted assessment of cfDNA concentration isolated from 4ml healthy donor plasma. Quantity was determined by Qubit fluorescent assay. The concentration of automated extraction using the QuantiDNA™ kit (Automated) was greater than the others.

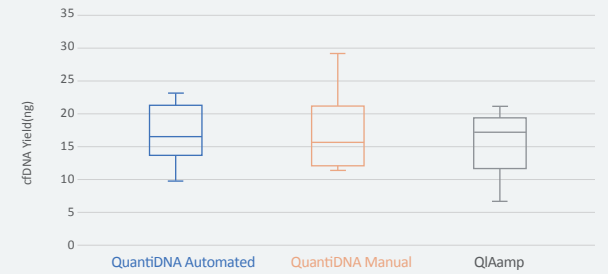


Figure 2. The box plot shows cfDNA yield from 4 ml of plasma. DNA concentrations were determined by Qubit fluorescent assay. cfDNA amount from QuantiDNA™ kits is comparable to the QIAamp kit.

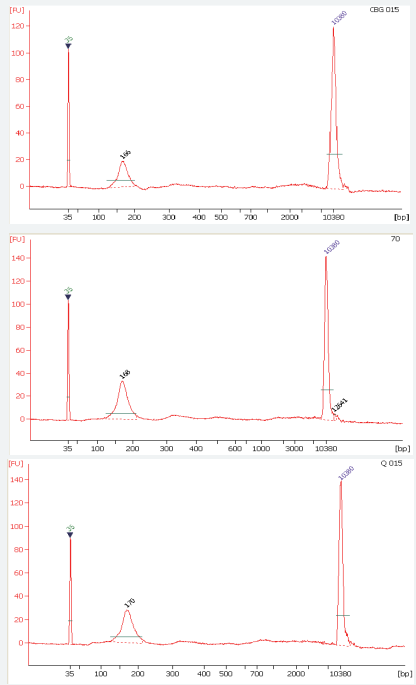


Figure 3. Bioanalyzer traces showing cfDNA integrity recovered from a healthy volunteer (a) QuantiDNA ™–Automated. (b) QuantiDNA™–Manual. (c) QIAamp.

Recovery Consistency and Inhibition Assessment

To evaluate the recovery consistency and inhibition possibility to downstream qPCR assay, plasma was prepared from the whole blood spiked with 1000 copies of 200 bp synthetic DNA fragment, gBlock, containing the KRAS G12 or APC R1450 mutation. cfDNA was extracted from 4 mL of these plasma using QuantiDNA™ kits (Automated and Manual) and QIAamp™ kits (manual). The amount of the recovered mutant fragment was evaluated using components of DiaCarta's ColoScape for targets KRAS G12 and APC1450 detection. The three kits showed similar Ct values in qPCR reactions for both KRAS G12 and APC R1450, suggesting that the spike-in gblocks are effectively extracted using both kits with similar yields (Figure 4).

To compare the qPCR performance using the cfDNA extracted using the QIAamp kit (Manual) and the QuantiDNA™ kit (Manual), 1.6 ng cfDNA was used as the template for beta-actin qPCR assay. No apparent difference in Ct value (Figure 5a) was found. Similarly, the automated extraction of cfDNA using QuantiDNA™ kit (Automated) also showed similar amplification results using BRAF as a reference gene (Figure 5b) when compared with the cfDNA extracted from the same QIAamp kit.

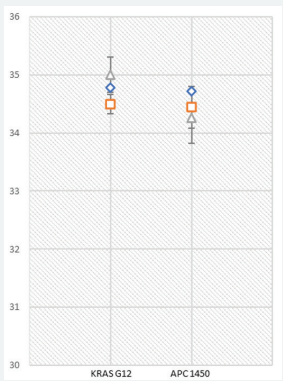


Figure 4. Extraction of cfDNA from plasma spiked with KRAS G12 or APC1450 gBlock followed by qPCR assays to evaluate the recovery of cfDNA and PCR inhibition possibility.

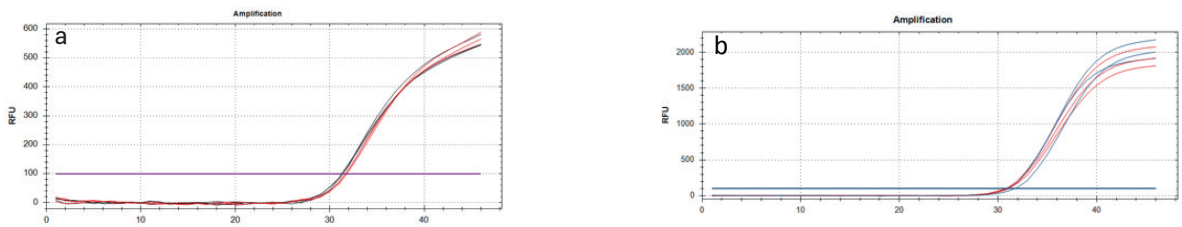


Figure 5. (a) Amplification of human housekeeping gene, Beta-actin. QuantiDNA – Manual (Red) compared to QIAamp (Blue) (b) Amplification of BRAF gene, QuantiDNA – Automated (Red) compared to QIAamp (Blue).

Order Information

Catalog Number	Product Name	Pack Size	Intended Use
DC-12-0002R	QuantiDNA™ Manual cfDNA Extraction Kit	50 Samples (for 4 ml plasma/sample)	Research use only
DC-12-0003R	QuantiDNA™ Automated cfDNA Extraction Kit	50 Samples (for 4 ml plasma/sample)	Research use only

SCAN TO LEARN MORE
MDC.21473