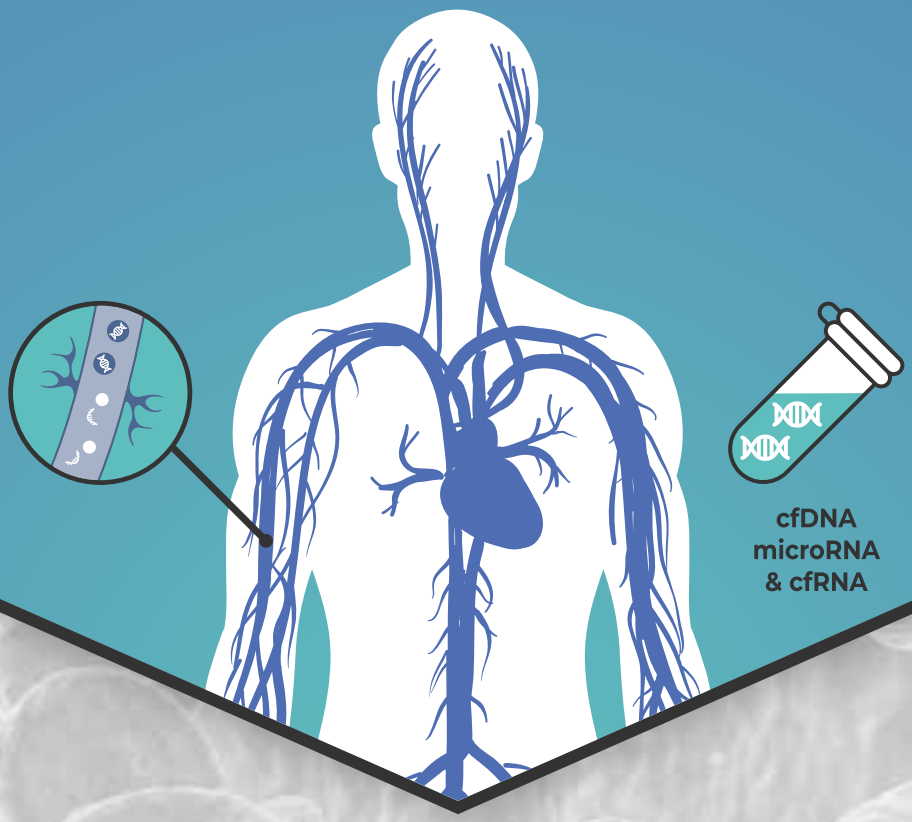
Purification & Quantification Circulating DNA-RNA



DANAGEN
Life Science Research

Cell-free Circulating DNA Purification

DANAGENE Circulating DNA Minikit provides a fast, reliable and convenient method to purify high quality, high purity and inhibitor-free cell-free circulating **DNA from fresh and frozen plasma / serum samples and other body fluids from samples of 1 ml** using a MicroSpin Columns specially developed to bind small fragments of DNA. OPTIONAL PROTOCOL for samples of 250-500 µl.

DANAGENE Circulating DNA Midikit provides a fast, reliable and convenient method to purify high quality, high purity and inhibitor-free **cell-free circulating DNA from fresh and frozen plasma / serum samples and other body fluids from samples of 3 ml** using a new column design for processing large volume sample volumes.

Specifications

- Efficient recovery and concentration of fragmented DNA (circulating cell-free DNA) with high input and low elution volume 30-35 µl.
- Sample size: Mini 1ml; Midi 3ml Midi fresh and frozen plasma/serum and other body fluids.
- No organic extraction or ethanol precipitation.
- Removal of contaminants and inhibitors.
- Yield: 0.1-100 ng / ml plasma or serum. Variable because each donor and disease status.
- Circulating DNA purified is ready for applications such PCR o real-time PCR, microarrays and Next generation sequencing.

Applications

- Biomarker research and validation for blood-based cancer detection.
- Ideal for detection of biomarkers in different diseases like autoimmune diseases, infection diseases, stroke, sepsis, trauma and hematologic disorders.
- Analysis of fetal DNA from maternal plasma.

New column design for processing large sample volumes.

Reference	Product Description	Preps
0614.1	DANAGENE Circulating DNA MINI Kit	50
0614.21	DANAGENE Circulating DNA MIDI Kit	5
0614.2	DANAGENE Circulating DNA MIDI Kit	50

Purification and quantification of circulating cell-free DNA from body fluids with DANAGENE Circulating System applied to Liquid Biopsy

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Introduction

The phenomenon of increased concentrations of circulating cell-free DNA (cfDNA) is considered a hallmark of various pathological conditions like cancer, autoimmune diseases, infectious diseases, stroke , sepsis, trauma and pregnancy.

Quantification of plasma cfDNA has been proposed as a diagnostic tool for cancer. The quantity of cfDNA is generally very low in healthy subjects (less than 5ng/ml of plasma) and increases (8 to 10 times) when considering subjects affected by a neoplastic disease, as well as in some physiological conditions.

Therefore, the development of reproducible, standaridzed methods for the detection and quantification of cfDNA is important for improving the sensitivity, specifity and relevance of this biomarker.

The isolation and quantification of cfDNA from body fluids represents a challenge, due to their small quantity and fragmented nature.

DANAGEN-BIOTED has developed a system for isolation of cfDNA for samples of 1 or 3 ml from body fluids using Midi Spin columns with a special resins that bound cfDNA.

Materials and Methods

Circulating Cell-Free DNA Isolation

Blood samples were collected from 8 patients (samples 1 to 8) with breast cancer and healthy controls. 2 samples were used for healthy individuals (sample 9 and 10) and 2 samples of healthy individuals were spiked with 150 ng (sample 11) and 300 ng (sample 12) of human genomic DNA. Plasma was carefully separated and stored at -80°C. The stored plasma was thawed at room temperature and centrifuged at 15.000 x g to remove residual precipitated cellular components. Circulating cell-free DNA was extracted from 3 ml of plasma following **DANAGENE Circulating DNA Kit** protocol.

Quantification of Circulating Cell-Free DNA

It was quantified the total amount of cf DNA isolated using the Cell-free human (cfh) DNA dtecqPCR Test developed by Genetic PCR Solutions™ (Alicante, Spain). The cfhDNA dtec-qPCR Test was designed to target a conserve sequence region of a gene repeated more than a hundred times in the human genome. qPCR, total volume of 20 ul qPCR mixture was prepared by adding 4 ul of MixStable qPCR.5x (GPSTM, Spain), 10 ul of nuclease free water, 1 ul of the primers/probe reagent (reagents included in the kit), and 5 ul of purified samples, following the instructions of the manufacturer. The real-time PCR thermal protocol used for amplification of the target gene, as recommended by the manufacturer, was: activation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 60 seconds. Data collection was performed during annealing/extension step by using the FAM channel.

Results

cfDNA were quantified by realtime PCR. The measured threshold cycle (Ct) and copies are listed in Table1. We successfully detected cfDNA in all cancer patients. Our results are consistent with others, and have shown that cfDNA can be detected in subjects with cancer.

Sample	Ct	Copies on assay	Sample concentration (copies ul)
1	22.34	6.8E+04	1.4E+04
2	21.18	1.4E+05	2.8E+04
3	20.67	2.0E+05	4.0E+04
4	22.21	7.4E+04	1.5E+04
5	22.43	6.4E+04	1.3E+04
6	20.82	1.8E+05	3.6E+04
7	23.30	3.6E+04	7.2E+03
8	21.33	1.3E+05	2.6E+04
9	26.31	5.0+03	1.0E+03
10	28.46	1.2E+03	2.4E+02
11	20.78	1.9E+05	3.8E+04
12	19.47	4.5E+05	9.0E+04

Table 1. Quantification of cfDNA from plasma samples

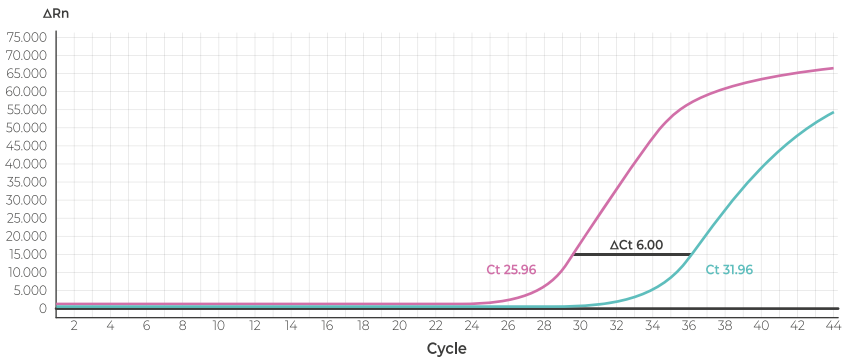

Figure 1. Real-time PCR amplification plot for cfhDNA dtec-qPCR Test (red) targeting a "nontruncated" multi-copy gene and compared to a monocopy target (blue), using a human genomic DNA as a standard. Due to the presence of multiple copies of the selected target, sensibility is increased 2 logs (100 times) for the cfhDNA dtec-qPCR Test. Same increased signal is observed for the purified cell-free DNA samples employed for cell-free DNA quantification.

Conclusion

The quantitative analysis of plasma DNA may be useful in distinguishing patients with cancer from healthy individuals.

In this paper, has been demonstrated the sensitivity in the quantification of cfDNA from plasma using DANAGENE Circulating DNA kit and Cell free human DNA dtec-qPCR Test.

This method will also be beneficial for isolating cfDNA in other pathological conditions. ctDNA collected without percutaneous tumor biopsy, also known as Liquid Biopsy, can become an innovative tool to analyze the cancer genome with obvious clinical importance for personalized treatment of cancer.

Cell-free Circulating RNA Purification

DANAGENE microRNA and Cell-free RNA Minikit/MidiKit provides an efficient isolation of **microRNA and small RNA from liquid biopsies including serum, plasma and others biofluids** without the use of toxic phenol or chloroform.

This kit allows to isolate all RNAs smaller than 1000 nt, from mRNA and tRNA down to microRNA and small interfering RNA (siRNA).

The sample material is denatured in Lysis buffer. Proteins are precipitated using the precipitation buffer and pelleted by centrifugation. After removal of proteins the binding conditions are adjusted by adding a **special Binding buffer** for small RNA. The small RNA are bound to special columns. The remaining RNAs are washed and eluted with minimal amounts of RNase-free water.

Specifications

- Efficient isolation of microRNA and Cell-free RNA from biofluids samples without phenol/chloroform.
- Sample size: Mini 300 µl (up to 600 µl multiple loading); Midi 3 ml fresh and frozen plasma/serum and other body fluids.
- Simple and fast procedure.
- Increased sensitivity in downstream applications.
- Yield: Depending on sample source, storage and quality.

Applications

- Ideal for detection of biomarkers in cancer and others diseases.
- Typical downstream applications: real-time qRT-PCR. Chip hybridisations.
- Analysis of fetal DNA from maternal plasma.

New column design for processing large sample volumes.

Reference	Product Description	Preps
0806.1	DANAGENE microRNA and Cell-free RNA MINI Kit	50
0806.2	DANAGENE microRNA and Cell-free RNA MIDI Kit	5
0806.3	DANAGENE microRNA and Cell-free RNA MIDI Kit	50

A system for miRNAs and cell-free RNA isolation from body fluids

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Introduction

miRNAs are small non-coding RNAs about 21–25 nucleotides in length. They are involved in RNA silencing and post-transcriptional regulation of gene expression and they have been found altered in the progression of different diseases. These molecules are found in most body fluids among them plasma and serum.

We quantified three different microRNAs. miR-223-3p has been detected in different experiments in our laboratory and is expressed in control samples at low CT values (around 20). miR-23a-3p and miR-451 are used to monitor sample hemolysis. miR-23a-3p CT value minus miR-451 CT value must be lower than 7 otherwise it is recommended not to use the sample.

DANAGEN-BIOTED has developed a system for isolation of microRNAs and cell-free RNA (cfRNA) for 300 µl (up to 600 µl multiple loading) of plasma or serum using Spin columns that bound RNA with <1000 nucleotides. The aim of the study was to compare different methods of microRNAs isolation.

Material and Methods

microRNA isolation

The plasma sample was collected from each patient and miRNAs were isolated using 4 different protocols: from 300 µl of sample with DANAGENE microRNA and Cell-free RNA Kit with and without carrier (MS2 RNA, Roche) and 200 or 300 µl of sample using a competitor kit and carrier MS2.

Universal reverse transcription and real-time PCR amplification

We use the miRCURY LNA™ Universal RT microRNA PCR (EXIQON). This protocol is based on universal reverse transcription (RT) followed by real-time PCR amplification using SYBR-Green. We used LNA™ enhanced primers specific for the three microRNAs. All reactions were performed in triplicate using a 7900HT device from Applied Biosystems.

Results

microRNAs were quantified by real-time PCR. The mean of the three threshold cycles (Ct) in each condition are shown in Figure 1. For all the miRNAs tested the Ct value was lower with the DANAGEN kit using the same sample volume. Ct values using the DANAGEN kit improved when a carrier was added to the sample.

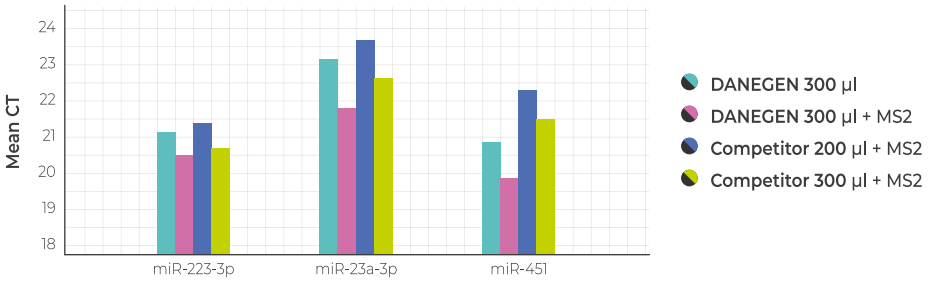

Figure 1. Mean Ct of 3 different reactions in each condition: Danagen kit w/o MS2 (300µl), Danagen kit w/ MS2 (300µl), competitor's kit w/ MS2 (200µl and 300µl)

Conclusion

This quantitative analysis of plasma miRNAs shows better results using DANAGENE microRNA and Cell-free RNA Kit. The results are further improved when using a carrier (MS2). In this paper we demonstrate that our kit can be used for an efficient isolation of small RNA from body fluids, useful for investigating miRNAs and cell-free RNA as circulating biomarkers for cancer and others diseases.

Quantification cfDNA

Quantification of cfDNA is ideally carried out by qPCR or capillary electrophoresis since common methods such as absorption measurement or fluorescent dye based quantification might lead to false results due to low DNA concentration.

The total cf DNA isolated can be quantified using the **Cell-free human DNA detc-qPCR** Test designed to target a conserve sequence region of a gene repeated more than a hundred times in the human genome.

Are individuals **ready-to-use tubes** containing all the components needed to perform the quantitative PCR assay.

Reference	Product Description	Preps
cfhDNA-24	cfhDNA MONODOSE detc.qPCR Test	24
cfhDNA-96	cfhDNA MONODOSE detc.qPCR Test	96

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