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QIAGEN GmbH – QIAamp Circulating Nucleic Acid Kit (55114) for Isolation of S-Monovette® cfDNA Exact stabilized samples

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Introduction

The biomarker cell free DNA (cfDNA) is playing an increasingly important role in research and diagnostics. For blood sample stabilisation SARSTEDT offers the innovative S-Monovette® cfDNA Exact ensuring excellent sample quality with a guaranteed stabilisation performance for 14 days at 4 - 37°C (Schrage et al. 2022)
Compatibility of blood collection tubes (containing cfDNA stabilizer solution) with isolation kits for cfDNA can be impaired due to the fact that cfDNA in the sample can be modified due to fixation by the stabiliser solution prefilled in the tubes. Therefore, we are pleased to show that S-Monovette® cfDNA Exact is compatible with a wide range of cfDNA isolation kits, in particular QIAamp Circulating Nucleic Acid Kit (55114) from QIAGEN GmbH as shown in the following application note. The details, and protocol optimizations, if necessary, are listed below.

Material and Methods

Blood sample collection and storage:

Blood from 3 healthy donors was drawn into S-Monovette® cfDNA Exact blood collection tubes (REF 01.2040.001, Sarstedt AG & Co., Nümbrecht, Germany) or S-Monovette® K3 EDTA (REF 01.1605.001, Sarstedt AG & Co., Nümbrecht, Germany).
Plasma was separated either within 1 hour after blood collection (D0) or after defined pre-analytical storage (D3).
For D3 the blood tubes were stored as follows: on day 0 after draw it was held at room temperature from the time of blood collection at 07:00 am, followed by sequential incubation phases of 30 °C (11:00 am), 40 °C (02:00 pm) and 25 °C (04:00 pm).
This 30 °C → 40 °C → 25 °C sequence was repeated at day 1 and day 2 (each at 11:00 am, 02:00 pm and 04:00 pm respectively) to simulate a blood sample transport in the summer time (profile corresponds to ISBT Guidance on Transport Validation). On day 3 at 11:00 am plasma was prepared.
Plasma separation was performed via two-step centrifugation as described in the cfDNA Exact Monovette manual / or protocol below. Plasma aliquots were stored at −80 °C until cfDNA isolation.

cfDNA isolation:

4ml of plasma from S-Monovette® cfDNA Exact and S-Monovette® K3 EDTA each at different time points were lysed and cfDNA was isolated as described in the manufacturers manual taking into account the input dependent scaling of the buffer volumes. A detailed protocol with the optimizations is attached.

Pre-analytics

cfDNA analysis:
To assess cfDNA quality, 2 µl of the eluate was analysed using the High Sensitivity cfDNA ScreenTape (Agilent) on the TapeStation system. The applicability of the isolated cfDNA to be used in common analysis methods was shown by using the samples in a qPCR assay to amplify the human endogenous retrovirus (***ERV-3***; Devonshire et al.,2014). The qPCR reactions were run with 5 µl sample input using the FastStart Universal SYBR® Green Master (ROX) (REF 04913914001, Roche Diagnostics GmbH, Mannheim) according to manufacturer's protocol on a Real-time Thermal cycler qTOWER3 (Analytic Jena GmbH, Jena, Germany). Primers (see Table 1) were used in a final concentration of 0.5 µM.

Table 1. Primer sequences

Primer	Sequence	Annealing temp.	Fragment length
ERV-3fw (Devonshire et al. 2014)	5'-CATGGGAAGCAAGGGAACTAATG-3'	60°C	135 bp
ERV-3rev	5'-CCCAGCGAGCAATACAGAATTT-3'		

Results & Discussion

Following the cfDNA isolation, quality control was carried out via a Tape Station (Agilent) with High Sensitivity cfDNA ScreenTape. Figure 1 shows the capillary electrophoretic separations of one donor. The stabilisation performance of the S-Monovette® cfDNA Exact becomes particularly clear here, since in contrast to storage of the S-Monovette® K3 EDTA, no release of genomic DNA can be detected on day 3. For the donor shown, the cfDNA peak has a low level, but this is not surprising as the donor belongs to a healthy control group.

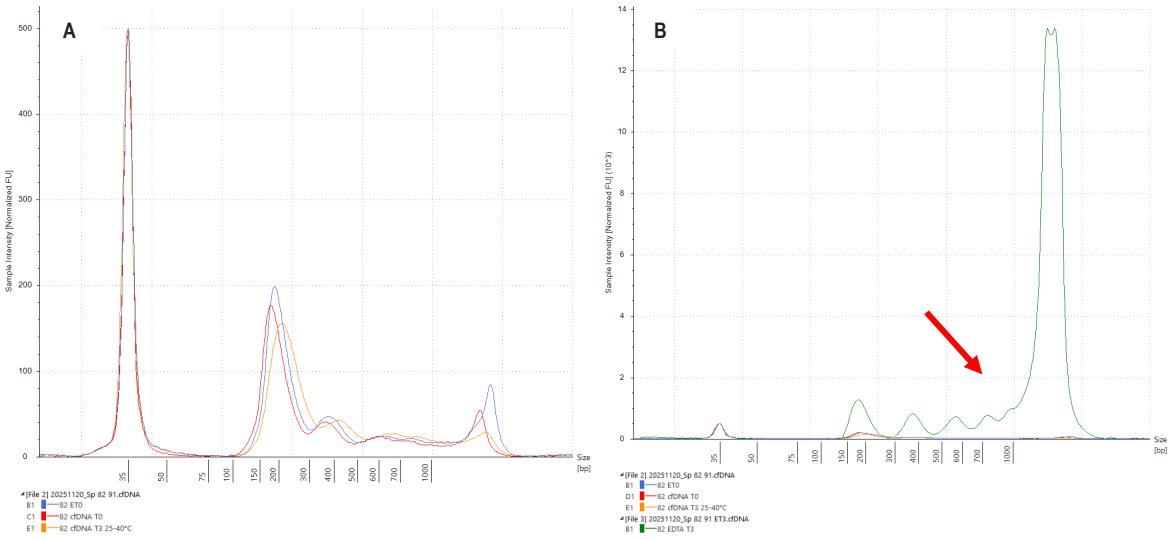

Figure 1: (B) Capillary electrophoretic representation of cfDNA using a Tape Station (Agilent). Illustration of one donor at the time point EDTA day 0 (blue), Day 0 in the S-Monovette® cfDNA Exact(red) and after 3 days(EDTA green/cfDNA Exact orange). The red arrow shows an entry of genomic DNA on day 3 into the sample from the S-Monovette® K3 EDTA (not stabilized samples); which is absent in the S-Monovette® cfDNA Exact due to stabilisation. (A) One can see a close up of the samples without the EDTA day 3 sample.

Subsequently, real-time PCRs of the isolated samples from 3 donors with one single-copy gene was performed. The C_T values of three time points are shown in figure 2A. Figure 2B shows the ΔC_T values calculated to the C_T value from the day 0 samples of the S-Monovette® K3 EDTA which was processed immediately after blood collection. Here, both the good isolation efficiency from the S-Monovette® cfDNA Exact and the stabilisation performance on day 3 at 25°C - 40°C become clear. No deviations beyond a donor-dependent variation could be detected.

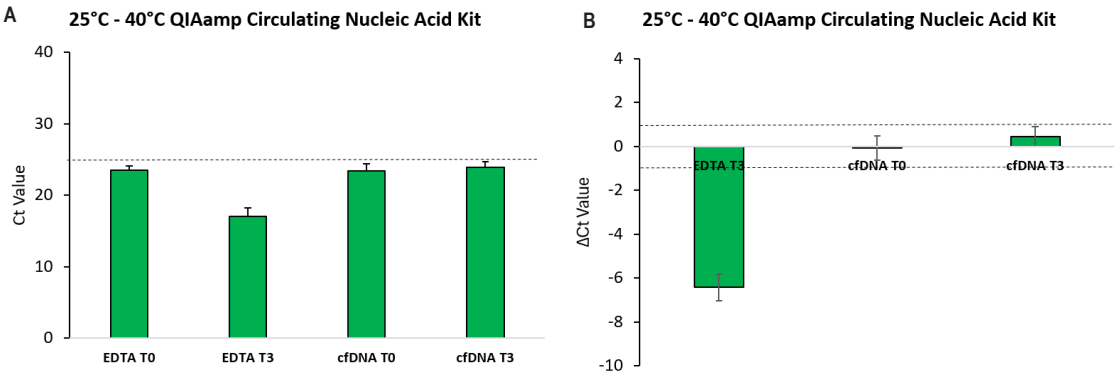

Figure 2: (A) C_T values from the tested single copy gene are shown at different time points. Average values from 3 donors with standard deviations were calculated. (B) Delta C_T value calculation for comparison to EDTA at time 0 were carried out. Values are shown averaged from 3 donors for a single copy gene. n=3

Summary

In this application note we show the compatibility of S-Monovette® cfDNA Exact stabilized samples with the QIAamp Circulating Nucleic Acid Kit (55114) from QIAGEN GmbH. With the kit, high quality cfDNA could be isolated following the manufacturers protocol. Small protocol adjustments which help to support the isolation are implemented in the protocol below. Additionally, it is shown that sample stabilisation of cfDNA samples is indispensable to achieve good sample quality.

Protocol

Prepare cell-free plasma samples from S-Monovette® cfDNA Exact

1. Centrifuge the blood samples at 2,000-3,000 x g for 10 minutes at room temperature
2. Transfer the plasma into a new centrifugation tube
3. Centrifuge the plasma samples at 15,000 x g for 15 minutes at room temperature
   (Alternatively, the plasma samples can be centrifuged at 6,000 x g for 30 minutes to remove any residual debris)

Lyse plasma samples with Proteinase K (PK)

1. Add the following components to a 15 ml tube (e.g. REF 62.554.100) in the order indicated

Reagents	Plasma volume
Proteinase K	400 µl
Plasma sample	4 ml
!Incubate for 5-10 min at room temperature and meanwhile prepare ACL buffer with Carrier RNA	
Buffer ACL with (1.0 µg Carrier RNA)	3.2 ml
Total volume	7.600 ml

2. Mix well and incubate at 60°C for 30 minutes on an Eppendorf™ ThermoMixer™ while gently shaking with 300 rpm
3. After PK digestion add 7.2 ml Buffer ACB and mix thoroughly by pulse-vortexing for 15–30 s.
4. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
5. Insert the QIAamp Mini column into a sufficient Vacuum pump. Insert a 20 ml tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

6. Carefully apply the lysate–Buffer ACB mixture from step 4 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender. Please note that large sample lysate volumes (about 20 ml when starting with 5 ml sample) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force. **Note:** To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.
7. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
8. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
9. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release pressure to 0 mbar.
10. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
11. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
12. Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube. Carefully apply 50 µl of Buffer AVE to center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min. **Important:** Ensure that the elution buffer AVE is equilibrated to room temperature.
13. Centrifuge in a microcentrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min to elute the nucleic acids.

Publication bibliography

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Notes

If you have any questions,
we'll be happy to help!

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