Zymo – Quick-cfDNA Serum & Plasma Kit (REF D4076) for Isolation of S-Monovette® cfDNA Exact stabilized samples

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 that S-Monovette® cfDNA Exact is compatible with a wide range of cfDNA isolation kits, and in particular compatibility with Quick-cfDNA Serum & Plasma Kit (REF D4076) from Zymo Research, as shown in the following application note. The details, and protocol optimisations, if necessary, are listed below.

MATERIAL & METHODS

Blood sample collection and storage:

Blood from four healthy donorsBlood from four 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 within one hour after blood collection (D0) or after storage of the blood collection tube for 3 days at 25°C (D3) by two step centrifugation as described in the cfDNA Exact Monovette´s manual or the protocol below. The plasma was stored at -80 °C until cfDNA isolation.

cfDNA isolation:

1ml 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 can be found below.

cfDNA analysis:

To assess the cfDNA quality 1 µl of the eluate was visualised electrophoretically on a High Sensitivity DNA Chip (Agilent REF 5067-4626) with the Bioanalyzer 2100. 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 single copy genes *myostatin* (*MSTN*; Breitbach *et al*., 2014) and a human *endogenous retrovirus* (*ERV-3*; Devonshire *et al.*,2014). The qPCR reactions were run with 8 µl sample input using the Maxima SYBR Green/ROX qPCR Master Mix (REF K0223, Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to manufacturer's protocol on a Mastercycler ep realplex 4S (Eppendorf, Hamburg, Germany) or Real-time Thermal cycler qTOWER^3 (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'		
MSTNfw (Breitbach *et al.* 2014)	5'-TTGGCTCAAACAACCTGAATCC-3'	60°C	88 bp
MSTNrev	5'-TCCTGGGAAGGTTACAGCAAG-3'		

RESULTS & DISCUSSION

Following the cfDNA isolation, quality control was carried out via an Bioanalyzer (Agilent) with the High Sensitive DNA Analysis Kit. Figure 1 shows the capillary electrophoretic separations of two donors. 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 donors shown, the cfDNA peak has a low level, but this is not surprising as the donors are a healthy control group.

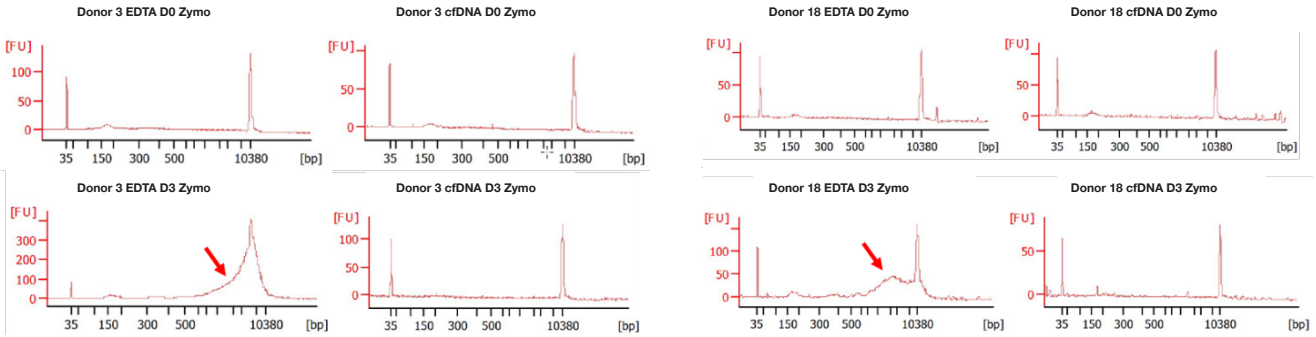

Figure 1: Capillary electrophoretic representation of cfDNA using a Bioanalyzer (Agilent). Illustration of two donors at the time point day 0 and after 3 days at 25°C. The red arrow shows an entry of genomic DNA on day 3 into the sample from the S-Monovette® K3 EDTA (not stabilized samples); which is absent in the S-Monovette® cfDNA Exact due to stabilisation.

Subsequently, real-time PCRs of the isolated samples from 4 donors with two single-copy genes were performed. The C_T values of the 4 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 (gold standard). Here, both the good isolation efficiency from S-Monovette® cfDNA Exact stabilized samples and the stabilisation performance on day 3 at 25°C become clear. No deviations beyond a donor-dependent variation could be detected.

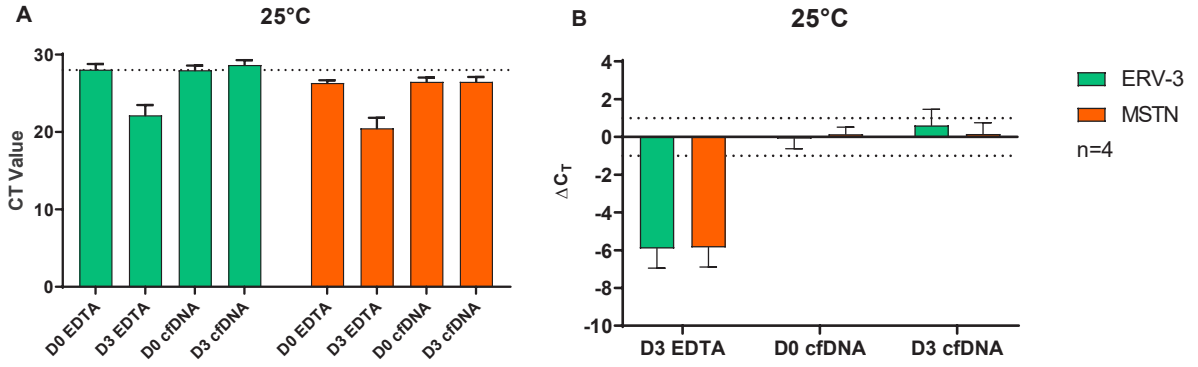

Figure 2: (A) C_T values from the tested single copy genes are shown at different time points. Average values from 4 donors with standard deviations were calculated. (B) Delta C_T value calculation for comparison to EDTA at time 0 were carried out. Values are shown averaged from 4 donors for two single copy genes with standard deviations.

SUMMARY

In this application note we show the compatibility of S-Monovette® cfDNA Exact stabilized samples with *Quick*-cfDNA Serum & Plasma Kit from Zymo Research. With the kit, high quality cfDNA could be isolated following the manufacturer´s protocol without substantially adjustments (see protocol below). Additionally, it is shown that sample stabilisation of cfDNA samples is indispensable to achieve good sample quality.

DETAILED ISOLATION PROTOCOL

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

1. **Replace the red screw-on lid of S-Monovette® cfDNA Exact with the centrifugation cap included**
2. Centrifuge the blood samples at 2.000-3.000 x g for 10 minutes at room temperature
3. Transfer the plasma into a new centrifugation tube (e.g. 5 ml reaction tube Sarstedt REF 72.701.400)
4. Centrifuge the plasma samples at 15.000 x g for 15 minutes at room temperature and transfer supernatant into a new tube. Continue to isolate cfDNA or store the plasma at -80°C until further use.

Scaling table for sample preparation

Plasma volume* [µl]	Proteinase K [µl]	Digestion Buffer [µl]	Binding Buffer [µl]
200	20	50	540
1.000	100	250	2.700
3.000	300	750	8.100

*For other sample volumes please scale according to the table in the kit´s manual

Lyse plasma samples

1. Submit 1.000 µl Plasma to a lysis tube with suitable volume (e.g. 5 ml reaction tube Sarstedt REF 72.701.400).
2. Add 100 µl Proteinase K.
3. Add 250 µl S&P 5X Digestion Buffer, mix
4. Place tube(s) into an suitable Thermo Mixer, heat up to 55°C with tubes placed inside, than incubate for 10 minutes while mixing (1.000 rpm).
5. Spin down to collect the fluid.

Adjust binding conditions

6. Add 2.700 µl S&P DNA Binding Buffer (two volumes) and mix
7. Spin down to collect the fluid

Bind DNA to column matrix (feasible with vacuum manifold or via centrifugation)

8. Load the lysate to the funnel of the Zymo-Spin™ III-S Column Assembly and apply vacuum until the lysate flowed through the column or centrifuge in 50 ml conical tubes at 1.000 x g for 2 minutes.

Wash silica membrane

9. Unscrew the cap from the top of the spin column and discard the funnel.
10. Place the spin column into a new collection tube (if not provided in sufficient amounts you may use the SARSTEDT Micro Tube REF 72.708)
11. Add 400 µl S&P DNA Prep Buffer
12. Centrifuge at 13.000 x g for 0.5 min and discard the flow through
13. Add 700 µl S&P Wash Buffer to the spin column and centrifuge at 13.000 x g for 0,5 min, discard the flow through
14. Add 400 µl S&P Wash Buffer and centrifuge at 13.000 x g for 1-2 min to ensure complete removal of the wash Buffer
15. Transfer the spin column in 1.5 ml DNase-free collection tube (e.g. SARSTEDT 72.704.400 or 72.704.200).

Elute the cfDNA

16. Add ≥50 µl DNA Elution Buffer.
17. Incubate for 5 min at room temperature.
18. Centrifuge at 11.000 x g for 1 minute and discard the spin column.
19. Store the eluted cfDNA at -20°C or below until further analysis.

Publication bibliography

Breitbach, Sarah; Tug, Suzan; Helmig, Susanne; Zahn, Daniela; Kubiak, Thomas; Michal, Matthias *et al.* (2014): Direct quantification of cell-free, circulating DNA from unpurified plasma. In PloS one 9 (3), e87838. DOI: 10.1371/journal.pone.0087838.

Devonshire, Alison S.; Whale, Alexandra S.; Gutteridge, Alice; Jones, Gerwyn; Cowen, Simon; Foy, Carole A.; Huggett, Jim F. (2014): Towards standardisation of cell-free DNA measurement in plasma. Controls for extraction efficiency, fragment size bias and quantification. In *Analytical and bioanalytical chemistry* 406 (26), pp. 6499–6512. DOI: 10.1007/s00216-014-7835-3.

Schrage, Kathrin; Linden, Justus; Kämper, Martin; Reiter, Jenny; Schuster, Rainer (2022): Comparison of S-Monovette® cfDNA Exact with two blood collection tubes for stabilization of cfDNA. SARSTEDT AG & Co. KG. Nümbrecht. Available online at https://www.sarstedt.com/fileadmin/user_upload/Mediacenter/Studien/Studie_neu/an_009_comparison_of_smonovette_cfdna_exact_0922.pdf.

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