Promega GmbH – Maxwell® RSC ccfDNA LV Plasma Kit (REF AS1840) 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 to show that S-Monovette® cfDNA Exact is compatible with a wide range of cfDNA isolation kits, in particular Maxwell® RSC ccfDNA LV Plasma Kit (REF AS1840) from Promega GmbH as shown in the following application note. The details, and protocol optimizations, if necessary, are listed below.

MATERIAL & METHODS

Blood sample collection and storage:

Blood from 15 healthy donors was drawn into S-Monovette® cfDNA Exact blood collection tubes (REF 01.2040.001, SARSTEDT AG & Co. KG, Nümbrecht, Germany) or S-Monovette® K3 EDTA (REF 01.1605.001, SARSTEDT AG & Co. KG, 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:

2 ml 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.

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 a Bioanalyzer 2100 (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.

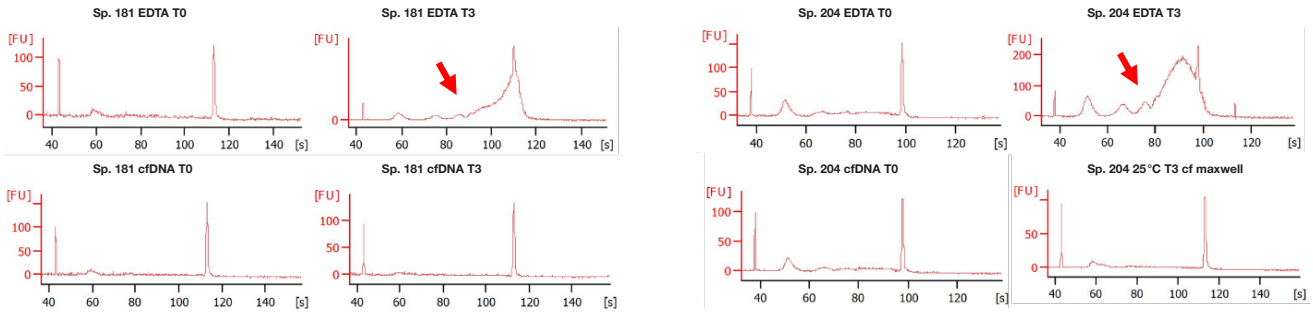

Figure 1: Capillary electrophoretic representation of cfDNA using a Bioanalyzer (Agilent). Illustration of two donors at the time point day 0 and after 3 days at 25 °C. The red arrow shows an entry of genomic DNA on day 3 into the sample from the S-Monovette® K3 EDTA (not stabilized samples); which is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

Subsequently, real-time PCRs of the isolated samples from 15 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. Here, both the good isolation efficiency from the S-Monovette® cfDNA Exact and the stabilisation performance on day 3 at 25°C become clear. No deviations beyond a donor-dependent variation could be detected.

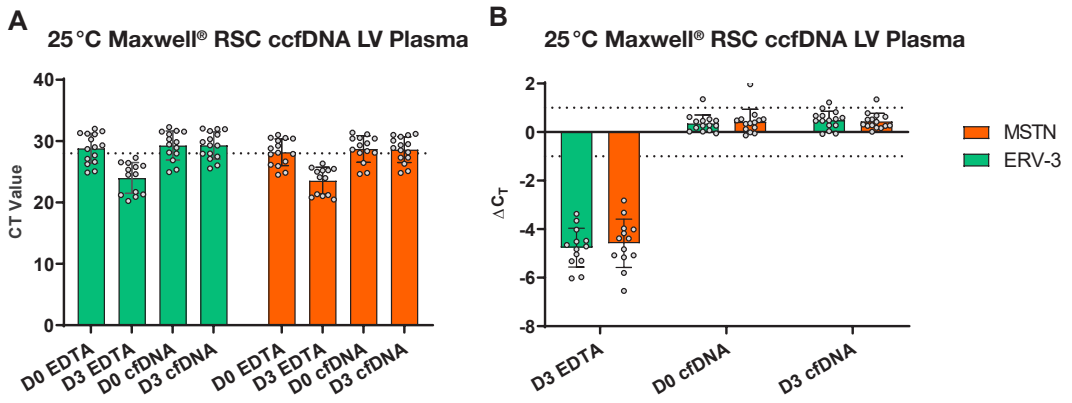

Figure 2: (A) C_T values from the tested single copy genes are shown at different time points. Average values from 15 donors with standard deviations were calculated. (B) Delta C_T value calculation for comparison to EDTA at time 0 were carried out. Values are shown averaged from 15 donors for two single copy genes with standard deviations.

SUMMARY

In this application note we show the compatibility of S-Monovette® cfDNA Exact stabilized samples with Maxwell® RSC ccfDNA LV Plasma Kit (REF AS1840) from Promega GmbH . With the kit, high quality cfDNA could be isolated following the manufacturers protocol with additional Proteinase K digestion. 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. **Replace the red screw-on lid 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 (for example SARSTEDT REF 72.701.400 or 62.554.100)
4. 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 5 ml/15 ml tube (e.g. REF 72.701.400/62.554.100) in the order indicated

Reagents	Plasma volume		
	2 ml	4 ml	6 ml
Proteinase K, 20 mg/ml (order separately e.g. Promega - REF MC5005)	100 µl	200 µl	300 µl
Plasma sample	2 ml	4 ml	6 ml
Binding Buffer	2 ml	4 ml	6 ml
Total volume	4.200 ml	8.400 ml	12.600 ml

2. Mix well and incubate at 56 °C for 60 minutes on an Eppendorf™ ThermoMixer™ while shaking with 1,000 rpm
3. After PK digestion add Maxwell® Resin E according to the following table and incubate for 45 min on a Rotisserie Shaker.

Reagents	Plasma volume		
	2 ml	4 ml	6 ml
Maxwell® Resin E	100 µl	100 µl	100 µl

Note: To save time, the two incubations can also be combined. Incubate for 1h at 56 °C and 1,000 rpm with low volume (2 ml plasma).

4. Centrifuge the tubes at 1,000 x g for 2 minutes to pellet the resin. Alternatively, a magnetic stand can be used to immobilize the resin prior to decanting.
5. Carefully decant the supernatant. While decanting, we recommend placing a magnet alongside the resin pellet in the tube to hold it in place.
   Proceed to Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges.

Preparing Maxwell® RSC ccfDNA LV Plasma Cartridges

1. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5 ml). Place the cartridges to be used the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Using a pipette, transfer 500 µl of well #1 (the large well) into the tube containing the magnetic resin pellet.
3. Resuspend the resin in the reduced volume. We recommend using a pipette for resuspending the resin because vortexing might cause resin to adhere to the upper sides of the tube.
4. Transfer the resin and liquid back to well #1 of each cartridge (well #1 is the largest well).
5. Place one plunger into well #8 of each cartridge.
6. Place an empty elution tube into the elution tube position for each cartridge in the deck tray.
   Add 75 µl of the appropriate Elution Buffer to the bottom of each elution tube. This will give a final elution volume of approximately 60 µl after processing.
7. Run the samples on the Maxwell® Instrument according to the manufacturer's instructions in the Maxwell® RSC ccfDNA LV Plasma Kit manual.

Publication bibliography

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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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