Sera-Xtracta™ Cell-Free DNA Kit

User Guide

gelifesciences.com

Table of Contents

1 Introduction

Product code

29437807

Important

Read these instructions carefully before using the product.

Intended use

The product is intended for research use only and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

Storage

Once reconstituted, store Proteinase K solution at 2°C to 8°C. All other kit components should be stored at room temperature (15°C to 30°C).

Expiry

For kit expiry date please refer to outer packaging label.

2 Components

Kit Contents

Pack size: 96 Purifications -based on input of 2.0 mL of plasma.

Product code for the kit: 29437807

Component	Amount
Proteinase K	2 x 60 mg (dried)
20% SDS	12 mL
Magnetic silica bead suspension	1.75 mL
Binding buffer	2 x 170 mL
Wash buffer 1 [1]	85 mL
Wash buffer 2 [1]	35 mL
Elution buffer	10 mL

[1] These components require ethanol addition prior to first use.

Materials to be supplied by user

- DNase-free water
- Absolute ethanol
- 2-propanol (isopropanol)

Equipment needed

- Centrifuges that accommodate 15 mL centrifuge tubes and 1.5 mL microcentrifuge tubes
- Standard laboratory shakers / mixers, for example the Eppendorf™ Thermomixer™ to accommodate 15 mL centrifuge tubes and 1.5 mL microcentrifuge tubes
- Incubator set to 60°C (can use Eppendorf Thermomixer if available)
- Vortex mixer
- 15 mL centrifuge tubes and 1.5 mL microcentrifuge tubes
- Pipette tips with aerosol barrier

- Magnetic racks, to fit 15 mL centrifuge tubes and 1.5 mL microcentrifuge tubes, e.g. MagRack 6 and MagRack Maxi

Note: *All tubes and pipette tips should be DNase-free grade. DNA Lo-Bind tubes are recommended. Good laboratory practices should be followed to avoid sample contamination.*

3 Description

Introduction

The **Sera-Xtracta Cell-Free DNA Kit** is designed for rapid extraction and purification of cell-free DNA (cfDNA) from blood plasma. The product has been designed to select for small fragment cfDNA over any higher molecular weight genomic DNA. It is recommended that blood is collected in Streck™ Cell-Free DNA BCT™ tubes.

The isolation procedure can be completed in less than 2 hours to yield high quality cell-free DNA suitable for downstream applications such as PCR, digital droplet PCR (ddPCR), genotyping and next generation sequencing (NGS).

The kit contains sufficient reagents to perform cfDNA extractions from 96 samples of 2 mL plasma.

The method uses chaotropic agents and detergents to disrupt protein components to which the cfDNA may be initially bound and promote the selective binding of this cfDNA to the silica magnetic beads. Proteinase K is used to digest protein and reverse Streck stabilization chemistry; denatured contaminants are easily removed by subsequent washing of the silica beads with specially formulated wash buffers.

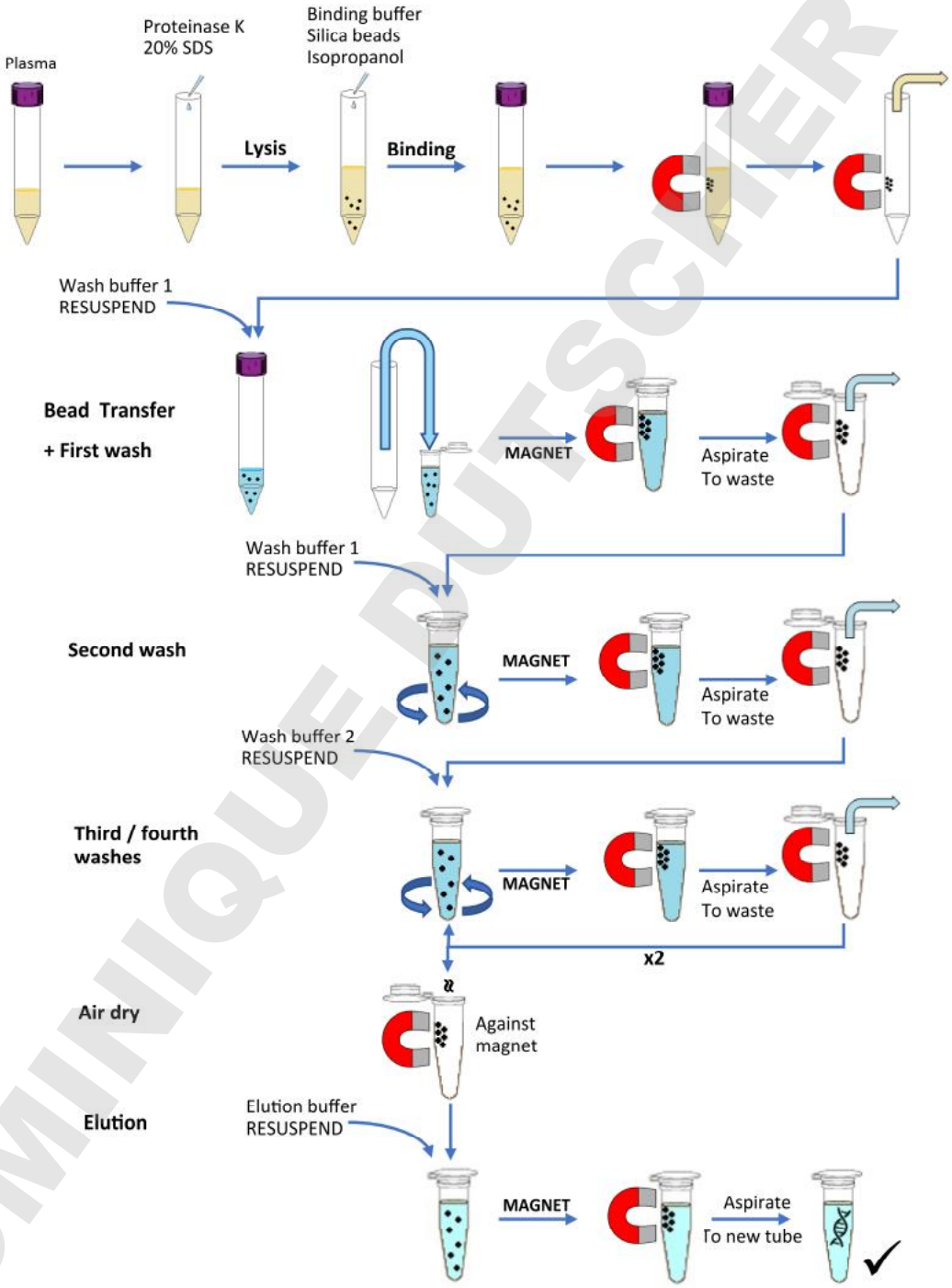
The purified cfDNA is eluted in a low ionic strength buffer, at a concentration suitable for most downstream molecular biology applications.

In this chapter

3.1 The basic principle

Illustrated procedure

Use of the **Sera-Xtracta Cell-Free DNA Kit** involves the following steps:



Step procedure

Step	Comments	Component
1. Lysis	Release of cfDNA from macromolecular complexes (reversal of Streck DNA stabilization chemistry)	Proteinase K 20% SDS
2. DNA Binding	Promotes selective binding of cfDNA to the magnetic silica beads. Denatured proteins/lipids are removed by aspiration of the cleared supernatant after magnet bead collection	Magnetic bead suspension Binding buffer Isopropanol
3. Bead transfer	Transfer from 15ml tube to 1.5 mL microtube	Wash buffer 1
4a. First wash	Wash buffer 1, containing a chaotropic salt, removes protein and other contaminants from bead-bound cfDNA	Wash buffer 1
4b. Additional washes	Wash buffer 2 containing ethanol removes residual Wash buffer 1 salts and other contaminants	Wash buffer 2
5. Dry	Excess ethanol from wash buffer 2 is removed by additional aspiration and air drying	--
6. Elution	The cfDNA is eluted from the beads using low ionic strength buffer and transferred into a fresh tube	Elution buffer

3.2 Product specifications

The **Sera-Xtracta Cell-Free DNA Kit** is recommended for the isolation of cfDNA from blood plasma collected in Streck Cell-Free DNA BCT tubes , amounts as indicated below.

Sample Type:	Plasma
Sample input volume	0.5 to 4.0 mL (standard input 2.0 mL)
Elution volume	30 µL[1]
Product size	Optimal for dsDNA 50 to 400 bp
Scalability	Up to 4.0 mL

[1] Recommended elution volume for a standard input 2.0 mL plasma: the end user has the option to amend as per downstream application requirements.

3.3 Typical output

Cell-free DNA

Cell-free DNA (cfDNA), also known as circulating cell-free DNA (ccfDNA), comprises small (< 500 bp) degraded fragments of genomic DNA that are released into blood.

cfDNA exists in plasma across a wide range of concentrations (e.g. 0.1 to 500 ng/mL of plasma). Levels at the lower end of this range are typical for healthy individuals, although reported to increase with ageing and temporarily post exercise. Elevated levels of cfDNA are observed in certain disease states, e.g. cancer.

cfDNA can also arise from fetal source in the blood of pregnant women and is routinely used for lower risk non-invasive prenatal testing in cases of suspected aneuploidy.

cfDNA extraction

The predominant type of cfDNA found in plasma is derived from the nuclear genome and has a fragment size that corresponds to a single nucleosome. These macromolecular complexes must be dissociated in order to allow for DNA binding to silica beads. In addition, some of the cfDNA is believed to be encapsulated in lipid vesicles and needs to be released prior to the binding step. The release of cfDNA from these diverse macromolecule complexes is achieved by a combination of detergents and protein digestion (proteinase K). In addition, proteinase K treatment allows to reverse the effects of Streck stabilization chemistry which would otherwise prevent efficient recovery of the cfDNA during the isolation process.

cfDNA concentration

As the levels of cfDNA encountered in blood plasma are very low, a significant volume reduction is needed during the isolation process to generate sufficient concentration for analysis. Efficient binding of the cfDNA from the sample, gentle washing and minimal elution volume are key to providing purified cfDNA that is suitable for downstream applications

Due to the low levels of cfDNA in the final extract, UV-absorbance based analysis is not recommended. cfDNA concertation should be evaluated using qPCR or fluorescence-based methods such as Qubit™ (Invitrogen™).

Qubit dsDNA HS Assay Kit, that is compatible with any fluorometer or fluorescence plate reader, allows for accurate estimation of total DNA concentrations down to 10 pg/µL (with somewhat lower sensitivity for shorter DNA fragments). Please also note that the concentration will represent total DNA content and in circumstances where some genomic DNA carry over is present, this measurement will misrepresent the actual content of cfDNA.

To assess the quality and yield of cell-free DNA in addition to the presence of gDNA, assessment can be performed using the Agilent 2100 Bioanalyzer system, with the Agilent High Sensitivity DNA Analysis Kit . Typical Bioanalyzer traces showing a major peak around 170 bp in Figure 3.1. The size range of the cfDNA peaks shown is typical for that observed from plasma obtained from blood of healthy human donors. However, cfDNA originating from cancerous tumours has been reported to be highly fragmented with sizes down to 50 bp or less. Please note that cfDNA levels are donor dependent and can vary substantially (Figure 3.1).

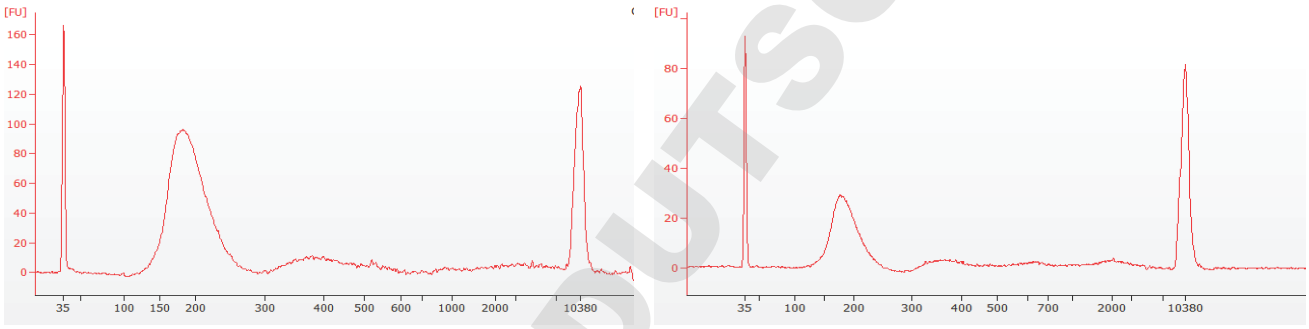


Figure 3.1: Typical Bioanalyzer traces showing cfDNA profiles recovered from two healthy volunteers . 2 mL of plasma obtained from blood collected in Streck Cell-Free DNA BCT tubes was processed using Sera-Xtracta Cell-Free DNA Kit and 1 uL was ran on a High Sensitivity DNA chip on the Bioanalyzer 2100.

4 Protocol

In this chapter

4.1 Preparation

Introduction

Numerous factors can affect the quantity and quality of the cfDNA isolated from blood plasma (these factors are outlined in detail in the appendix). Sera-Xtracta Cell-Free DNA Kit was specifically designed to ensure maximum recovery of cfDNA from blood plasma collected in Streck Cell-Free DNA BCT tubes.

Pre-use reagent checks

20% SDS, Binding buffer and Wash buffer 1.

Components of these reagents may crystalize/precipitate during shipment and storage. Should this occur, re-dissolve by gentle warming at 37°C (allow the reagents to equilibrate back to ambient temperature before use).

Preparation of working solutions

See *Chapter 2 Components, on page 4* for Materials and Equipment to be supplied by user.

Proteinase K (20 mg/mL):

Proteinase K is supplied in 2 vials at 60 mg. Prior to use, reconstitute to 20 mg/mL with 3 mL of DNase-free water. Vortex mix to dissolve and store the solution at 2°C– 8°C. Please note that reconstituted Proteinase K solution is stable for at least 4 months at 2°C to 8°C (to ensure maximum shelf life reconstitute each vial when needed).

Wash buffer 1

Prior to use, add 85 mL of absolute Ethanol to the Wash buffer 1 bottle. Mix by inversion. Indicate on the label that this step has been completed. Store upright and air tight at room temperature (15°C– 30°C).

Wash buffer 2

Prior to use, add 140 mL of absolute Ethanol to the Wash buffer 2 bottle. Mix by inversion. Indicate on the label that this step has been completed. Store upright and air tight at room temperature (15°C–30°C).

4.2 Sample collection and processing for plasma

In order to ensure the highest quality and quantity of cfDNA, the blood sample should be collected in Streck Cell-Free DNA BCT tubes. Separate the plasma from blood using the following protocol:

- After blood collection, store the collection tube(s) at ambient temperature until processing plasma as per manufacturer recommendation. Please note that all experiments presented in this document were performed on plasma processed within 7 days after blood draw.
- Centrifuge the collection tube(s) at 1600 × g for 10 min at 20°C.
- Aspirate the upper plasma fraction (approx. 4 – 5 mL per 10 mL blood) into a fresh tube (take care not to disturb buffy coat layer that is positioned between plasma and red blood cell layer).
- Re-centrifuge at 16 000 × g for 10 min at 20°C.
- Aspirate the cleared plasma fraction into a fresh tube leaving any cellular residue behind. For larger volumes of plasma and to ensure sample homogeneity, pool the individual aspirations, then re-aliquot into convenient units for cfDNA isolation e.g. 2.0 mL units.
- Process plasma for cfDNA isolation immediately or store aliquots at -20°C/-80°C until required.

Follow the protocol in *Section 4.3 Protocol for purification of cell-free DNA from 1.0–4.0 mL plasma, on page 15* for plasma volumes 1 – 4 mL. When purifying cfDNA from 0.5 mL of plasma, please refer to *Section 4.4 Protocol for purification of cell-free DNA from 500 µL plasma, on page 20*,

4.3 Protocol for purification of cell-free DNA from 1.0–4.0 mL plasma

Important

A pulse spin in a microcentrifuge is strongly recommended before magnet settling, to ensure all the liquid sample in the tube is collected together in a single volume at the bottom of the tube. Isolated droplets on the tube wall or trapped under the tube lid will not magnet settle / aspirate properly.

Illustrated procedure

For illustrations of the basic principle, see *Section 3.1 The basic principle, on page 7*.

Lysis

Step	Action
1	Add **Proteinase K** (20 mg/mL) and the input plasma sample into the bottom of a 15 mL microcentrifuge tube and mix by vortexing.
2	Add **20% SDS** to the sample from step 1.

Reagent	Plasma input volume		
	1 mL	2 mL	4 mL
Proteinase K	20 µL	40 µL	80 µL
20% SDS	50 µL	100 µL	200 µL

Note:

Either the Proteinase K or the plasma may be added to the tube first. Do not allow 20% SDS to contact the Proteinase K solution directly to prevent enzyme inactivation.

3	Pulse vortex 2-3 times and mix thoroughly by vortexing for 15 s.
4	Incubate the tube at 60°C for 20 minutes.

Binding mix preparation

Make sure that the magnetic beads are fully resuspended by vortexing before dispensing.

Prepare the **Binding mix** by combining the following (the relative amount of each component in the mix is critical for the maximum cfDNA recovery and minimum binding of genomic DNA; we recommend reverse pipetting to ensure volume accuracy).

Reagent	Plasma input volume		
	1 mL	2 mL	4 mL
Binding buffer	1.45 mL	2.90 mL	5.80 mL
Magnetic beads	7.5 µL	15 µL	30 µL
Isopropanol	0.70 mL	1.40 mL	2.80 mL

1. **Binding buffer** × (no of samples to be processed + 10%)
2. **Isopropanol** × (no of samples to be processed + 10%)
3. **Magnetic bead** suspension (no of samples + 10%)

Mix thoroughly by pulse vortexing.

This table illustrates the calculation of Binding mix components for 8 × 2mL plasma.

Reagents	Starting volume / 2 mL of plasma	No of samples	Calculation (no of samples +10%)	Volume in the Binding mix
Binding buffer	2.90 mL	8	2.9 mL × 8.8	25.52 mL
Magnetic beads	15 µL		15 µL × 8.8	132 µL
Isopropanol	1.40 mL		1.4 mL × 8.8	12.32 mL

Binding

Step	Action
1	Add the freshly prepared **Binding mix** to the tube (reverse pipetting recommended).
2	Mix thoroughly by pulse vortexing.

Reagent	Plasma input volume		
	1 mL	2 mL	4 mL
Binding mix	2.1 mL	4.2 mL	8.4 mL

3	Incubate the tube in the thermomixer (25°C, 1000 rpm) for 10 min.
4	Briefly spin and place the 15 mL tube into a magnet rack for at least 5 min.
5	The beads contain bound cfDNA and once they collect to the magnet, carefully aspirate the cleared supernatant to waste.

Bead transfer to 1.5 mL tubes

Step	Action
1	Remove the tube from the magnet rack.
2	Add 400 µL of **Wash buffer 1** to the tube directly to the bead pellet.
3	Fully resuspend the beads by pulse vortexing, briefly spin.
4	Pipette up and down and transfer the content of the 15 mL tube to a 1.5 mL microtube (due to the liquid viscosity, expel the content of the tip slowly to ensure complete transfer of the bead suspension).
5	Add a second aliquot of **Wash buffer 1** (400 µL) to the 15 mL tube, vortex, briefly spin and transfer the content to the corresponding 1.5 mL tube from the previous step.
6	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
7	Discard the supernatant.

Bead washes

Step	Action
1	Remove the tube from the magnet rack and add 700 µLof **Wash buffer 1** to the tube.
2	Incubate the tube in the thermomixer (25°C, 1400 rpm) for 1 min.
3	Vortex and briefly spin.
4	Place the tube into the magnet rack for 1min.
5	Discard the supernatant.
6	Remove the microtube from the magnet and add 700 µL of **Wash buffer 2**.
7	Incubate in a thermomixer at 25°C, 1400 rpm for 1 min.
8	Vortex and briefly spin.
9	Place the microtube into the magnet rack for 1 min.
10	Discard the supernatant.
11	Repeat steps 6-10.

Air dry

Step	Action
1	Briefly spin to collect any residual Wash buffer 2 at the bottom of the tube.
2	Place the tube into the magnet rack and allow 1 min for the beads to collect against the magnet.
3	Carefully remove the cleared residual supernatant from the very bottom of the microtube using a small pipet tip.
4	Allow the bead pellet to air-dry for 5 min while on the magnet rack.

Elution

Step	Action
1	Remove the tube from the magnet rack.
2	Add **Elution buffer** to the tube. Mix well by vortexing, to ensure the bead pellet is fully resuspended.

Reagent	Plasma input volume		
	1 mL	2 mL	4 mL
Elution buffer	15 µL	30 µL	60 µL

3	Incubate the tube in the thermomixer at 25°C, 1400 rpm for 3 min.
4	Briefly spin to bring the sample to the bottom of the tube.
5	Place the tube into the magnet rack and allow 1 min for the beads to collect against the magnet.
6	Once the beads collect to the magnet carefully transfer the supernatant containing the isolated cell-free DNA into a fresh microtube.

4.4 Protocol for purification of cell-free DNA from 500 µL plasma

The process can be carried out in a single 2.0 mL microtube.

Lysis

Step	Action
1	Add 10 µL of **Proteinase K** (20 mg/mL) and 500 µL of input plasma sample into a 2 mL microcentrifuge tube and mix by brief vortexing.
2	Add 25 µL of 20% SDS to the sample from step 1. ***Note:*** *Either the Proteinase K or the plasma may be added to the tube first. Do not allow 20% SDS to contact the Proteinase K solution directly to prevent enzyme inactivation.*
3	Pulse vortex 2-3 times and mix thoroughly by vortexing for 15 s.
4	Incubate the tube at 60°C for 20 min.

Binding mix prepararation

Make sure that the magnetic beads are fully resuspended by vortexing before dispensing.

Prepare the **Binding mix** by combining the following (the relative amount of each component in the mix is critical for the maximum cfDNA recovery and minimum binding of genomic DNA; we recommend reverse pipetting to ensure volume accuracy)

1. 0.725 mL **Binding buffer** × (no of samples to be processed + 10%)
2. 0.35 mL **Isopropanol** × (no of samples to be processed + 10%)
3. 3.75 µL **Magnetic bead** suspension (no of samples + 10%)

Mix thoroughly by pulse vortexing.

Binding

Step	Action
1	Add 1.05 mL of the freshly prepared **Binding mix** to the tube (reverse pipetting recommended).
2	Mix thoroughly by pulse vortexing.
3	Incubate the sample in the thermomixer (25°C ,1400 rpm) for 10 min.
4	Briefly spin and place the 2 mL tube into a magnet rack for at least 5 min.
5	The beads contain bound cfDNA and once they collect to the magnet, carefully aspirate the cleared supernatant to waste.

Washes × 4

Step	Action
1	Remove the tube from the magnet rack.
2	Perform washing steps using 700 µL of the wash buffers as follows:

Wash round	Wash buffer ID
1st	**Wash buffer 1**
2nd	**Wash buffer 1**
3rd	**Wash buffer 2**
4th	**Wash buffer 2**

3	Incubate the tube in the thermomixer (25°C, 1400 rpm) for 1 min,
4	Vortex and briefly spin.
5	Place the tube into the magnet rack for 1 min.
6	Discard the supernatant.
7	Repeat steps 1-6 using the indicated wash buffer for each round.

Air dry

Step	Action
1	Briefly spin to bring any residual wash buffer droplets to the bottom of the tube.
2	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
3	Carefully remove the cleared residual supernatant from the very bottom of the microtube using a small pipet tip.
4	Allow the bead pellet to air-dry for 5 min while in the magnet rack.

Elution

Step	Action
1	Remove the tube from the magnet rack.
2	Add 15 µL of **Elution buffer** to the tube and mix well by vortexing, to ensure the bead pellet is fully resuspended.
3	Incubate the tube in the thermomixer (25°C, 1400 rpm) for 3 min.
4	Briefly spin to bring the sample to the bottom of the tube.
5	Place the tube into the magnet rack and allow 1 min for the beads to collect against the magnet.
6	Once the beads collect to the magnet carefully transfer the supernatant containing the isolated cell-free DNA into a fresh microtube.

4.5 Storage of purified cell-free DNA

Purified cell-free DNA may be stored at 2°C-8°C for a short period if being used directly for analysis and/or downstream molecular biology applications.

For longer periods of storage, we recommend -20°C or -80°C .

The Elution buffer provided is compatible with the majority of downstream applications. However, the end user may substitute this for molecular grade water, if necessary.

Note: *Although DNase-free water alone can be used for elution, DNA eluted in water is not well suited for prolonged storage due to increased risk of acid hydrolysis.*

5 Appendices

In this chapter

5.1 Manual process typical result

The Bioanalyzer plot below show typical result obtained from the same plasma in three independent experiments.

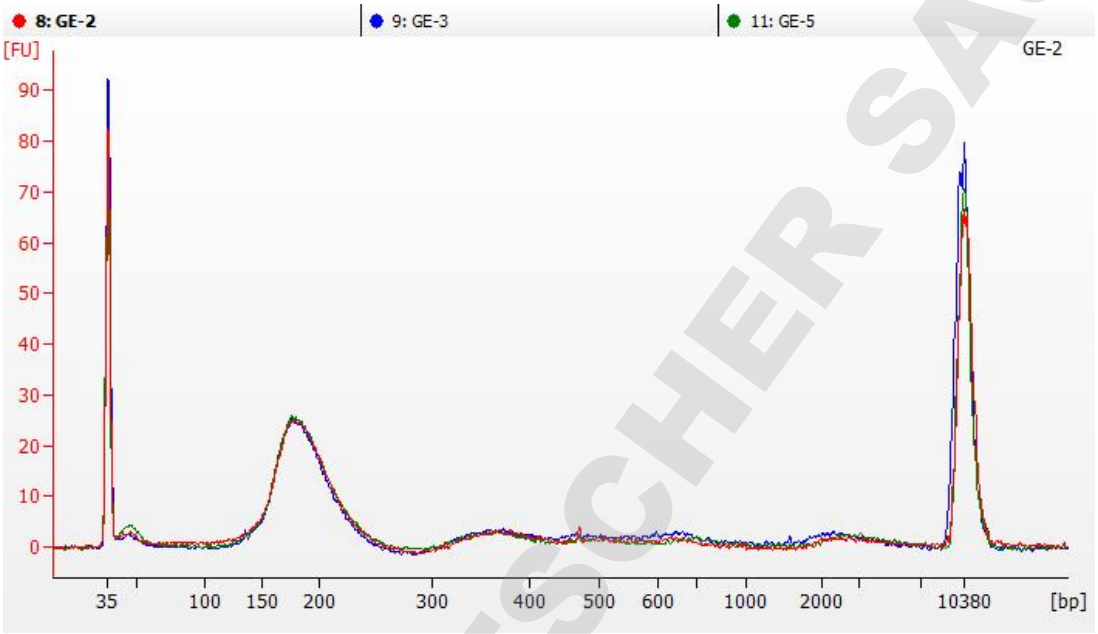


Figure 5.1: Typical Bioanalyzer traces showing cfDNA profile recovered from a healthy volunteer in 3 independent experiments (green, blue and red trace). 2 mLof plasma obtained from blood collected in Streck Cell-Free DNA BCT tubes was processed using Sera-Xtracta Cell-Free DNA Kit and 1 µL was ran on a High Sensitivity DNA chip on the Bioanalyzer 2100.

5.2 Scalability

The Bioanalyzer plots below show the results achieved for varying plasma input volumes (0.5 mL, 1 mL and 4 mL) compared to a standard 2 mL input. The extraction volumes have been scaled to the input volume, for comparable DNA concentrations in the extracts. Please note that due to potential issues with handling volumes below 15 µL we would generally recommend using 15 µL elution volume rather than 7.5 µL for 0.5 mL plasma input.

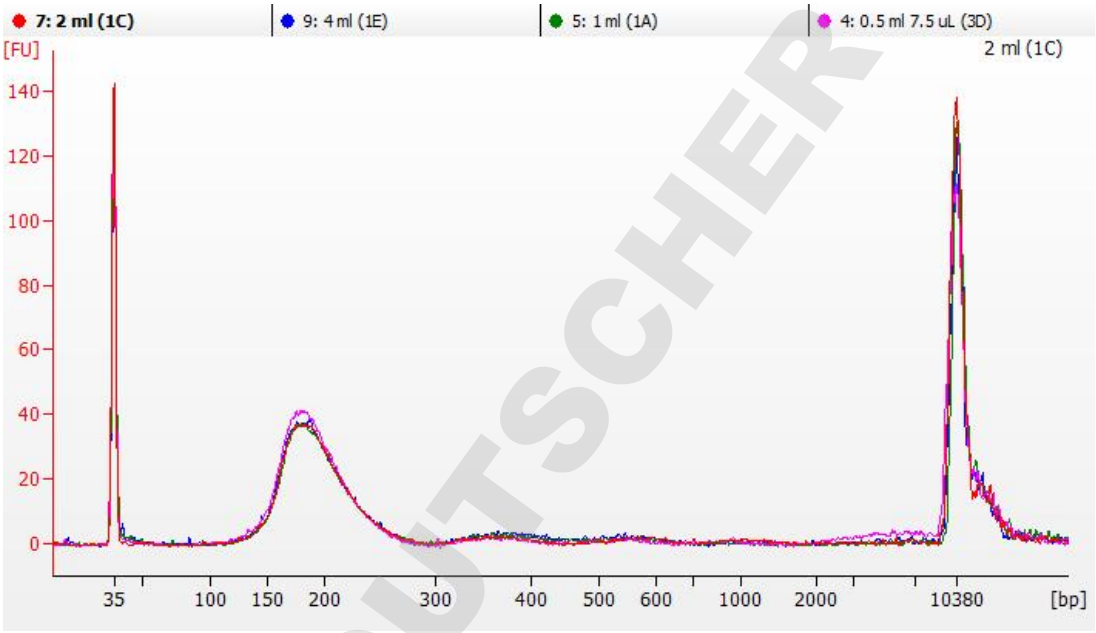


Figure 5.2: Bioanalyzer plots for 4 different plasma input volumes as described in the graph legend. Plasma obtained from blood collected in Cell-Free DNA BCT tubes was processed using Sera-Xtracta Cell-Free DNA Kit ; 1 uL was run on High Sensitivity DNA chip on the Bioanalyzer 2100.

Plasma input volume	0.5 mL	1.0 mL	2.0 mL	4.0 mL
Elution buffer volume	15 µL (7.5 µL)	15 µL	30 µL	60 µL

5.3 Recovery versus DNA fragment size

Sera-Xtracta Cell-Free DNA Kit has been designed to maximize the recovery of small degraded cfDNA fragments that have been reported to be present in plasma of patients with advance stage cancer and to represent a fraction enriched in DNA of tumor origin. At the same time the kit allows to considerably reduce co-purification of high molecular weight DNA representing gDNA originating from lysed blood cells. These two unique features of the kit are well illustrated in the Figure below which shows a size dependent recovery of 50 bp ladder fragments.

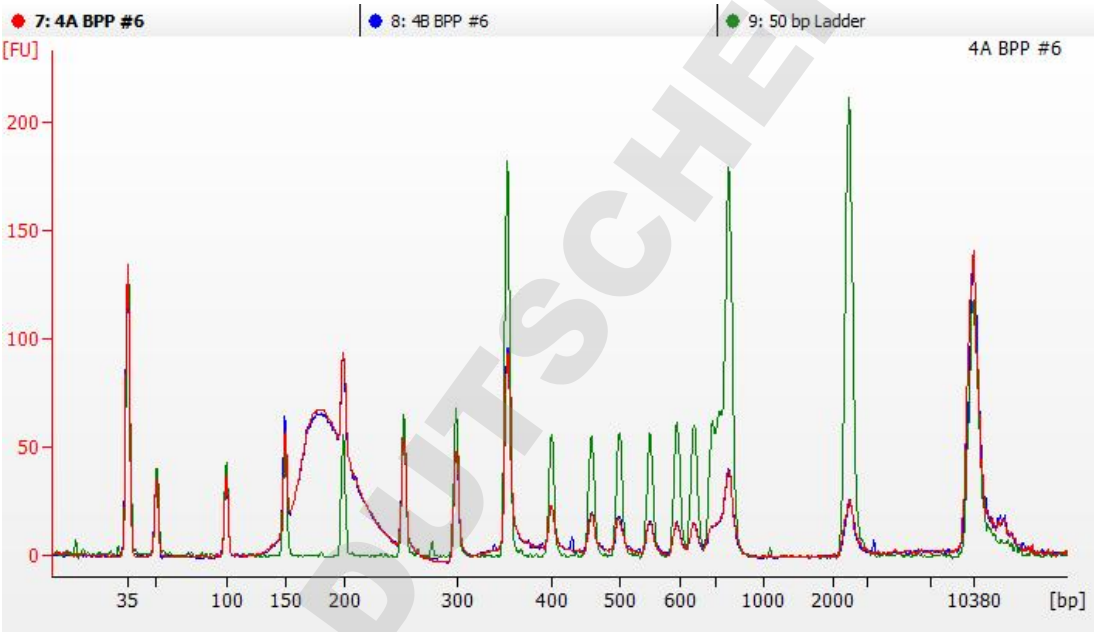


Figure 5.3: Bioanalyzer plots showing size dependent recovery of a 50 bp ladder fragments used to spike a healthy volunteer plasma (two independent extractions; blue and red trace respectively, ladder input in green). 2 mL of plasma obtained from blood collected in Streck Cell-Free DNA BCT tubes was spiked with 50 bp DNA ladder (10 ng/mL of plasma) and processed using Sera-Xtracta Cell-Free DNA Kit ; 1 µL was ran on High Sensitivity DNA chip alongside 50 bp DNA ladder input.

Expected recovery profiles for low molecular weight fragments , i.e. 50 bp and 100 bp and a high molecular weight fragment of 2.5 kb are presented in the Figure below.

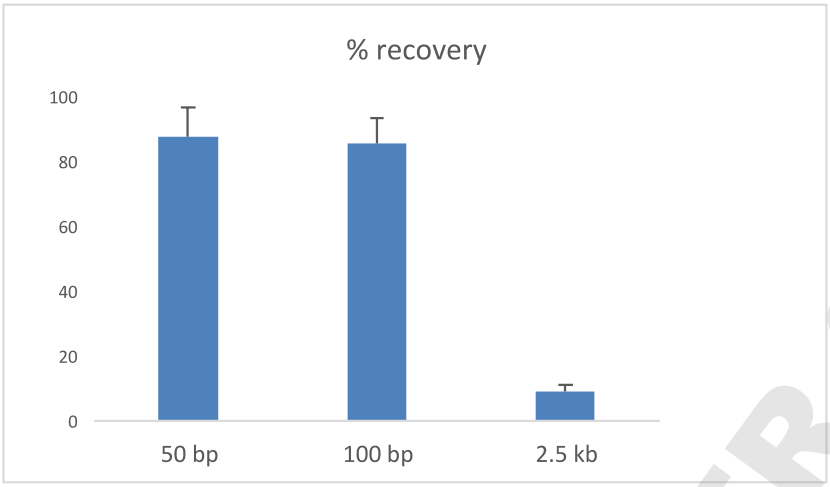


Figure 5.4: Percent recovery of spiked-in DNA ladder for selected fragments, based on 8 independent experiments (plasma obtained from 2 different healthy donors' blood collected in Streck Cell-Free DNA BCT tubes), error bars represent standard deviation.

5.4 Comparison, (semi)-automated versus manual processes

Automation of Sera-Xtracta Cell-Free DNA kit on the KingFisher™ Duo Prime instrument has also been demonstrated. An automation script[3] was developed for the Sera-Xtracta Cell-Free DNA kit based on the manual protocol. Cell-free DNA isolated on the KingFisher Duo Prime was analysed on Bioanalyzer and compared to cfDNA isolated from the same sample, using the manual protocol, see figures below.

- Plasma input volume was 2 mL.
- Wash reagents pre-loaded into deep-well plate before process.
- Elution buffer pre-loaded into elution strip before process.
- Freshly prepared binding mix was added manually to lysed plasma – all remaining steps are automated without further user input.

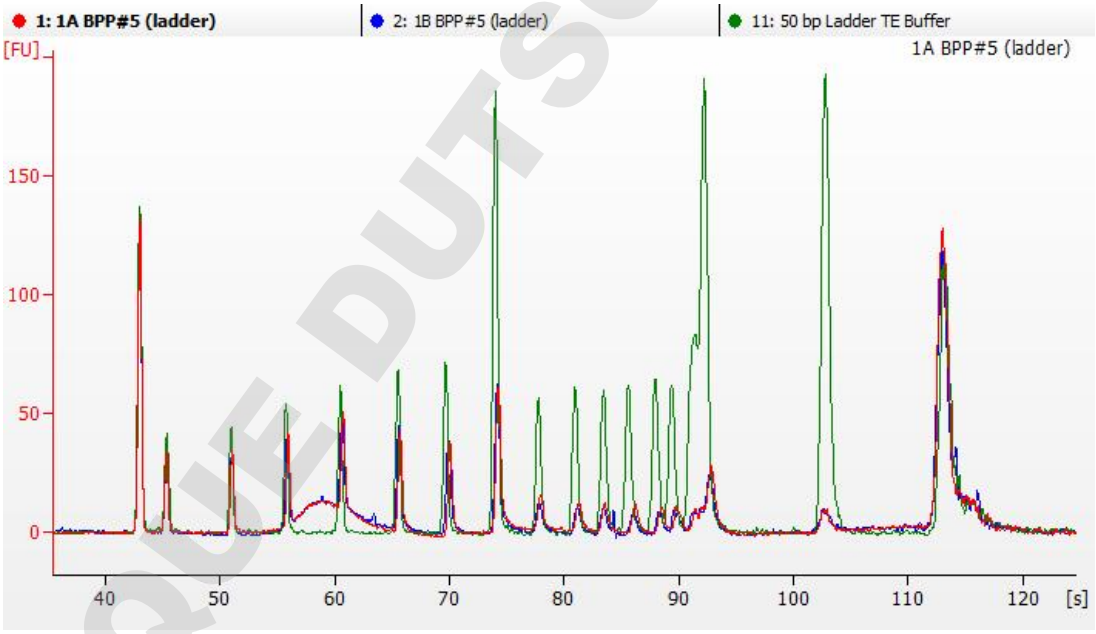


Figure 5.5: Example Bioanalyzer traces, with 2 mL plasma (obtained from blood collected in Cell-Free DNA BCT collection tubes (Streck) extracted in duplicates on KingFisher Duo Prime, spiked with a 50 bp DNA ladder (10 ng per mL of plasma).

3 An automation script for Kingfisher Duo is available on request from Scientific Support (https://www.gelifesciences.com/support/contact-us)

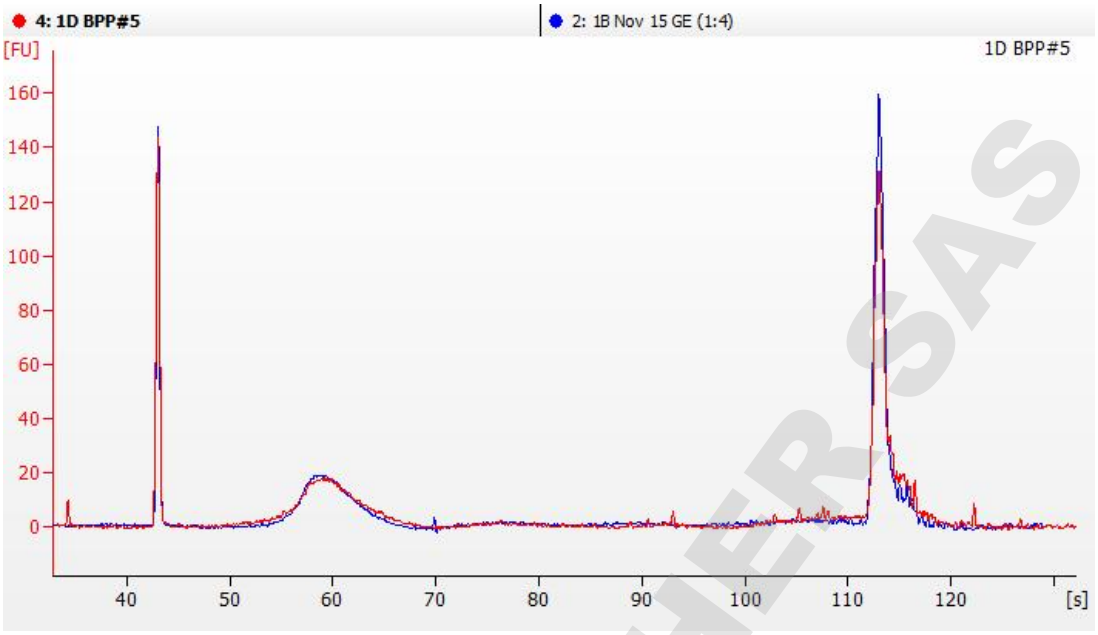


Figure 5.6: Comparison between manual (blue trace) and semi-automated (red trace) cfDNA extraction. Plasma (2 mL) obtained from blood collected in Cell-Free DNA BCT collection tubes (Streck), was processed using Sera-Xtracta Cell-Free DNA Kit and 1 µL was run on a High Sensitivity DNA chip on the Bioanalyzer 2100.

5.5 Expected result from plasma collected in standard EDTA blood collection tubes

The Sera-Xtracta Cell-Free DNA Kit has been validated for extraction of cfDNA from blood plasma collected in Streck Cell-Free DNA BCT tubes. It is possible to efficiently extract cfDNA from blood plasma collected in standard EDTA tubes which is illustrated in the Figure below. Please note that in these instances, the recovery of the smaller fragments might fall below levels expected for Streck Cell-Free DNA BCT tubes.

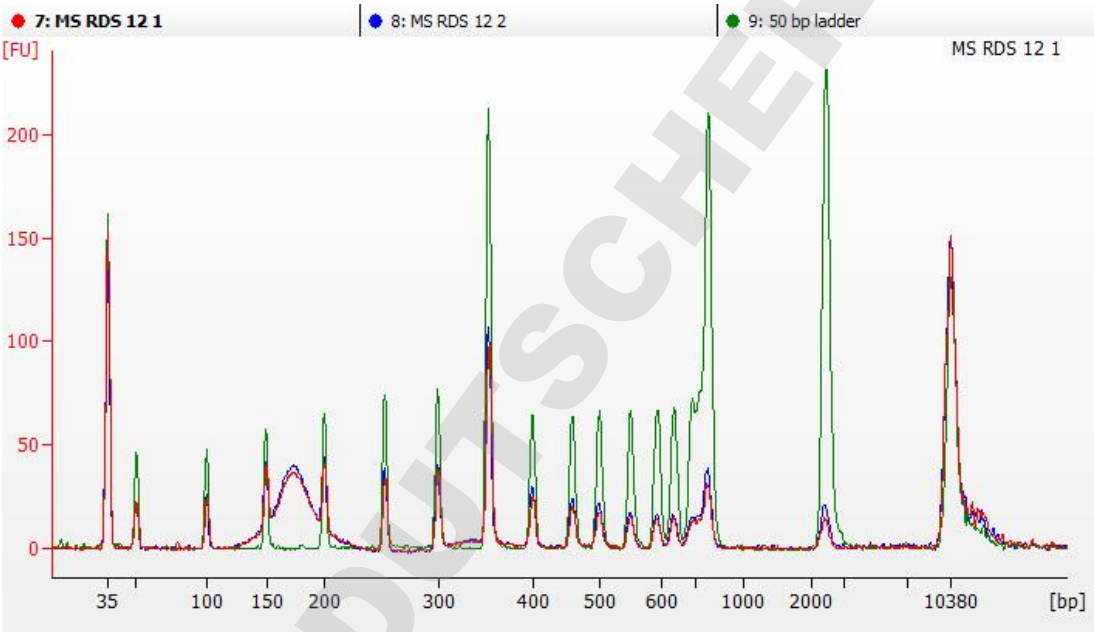


Figure 5.7: Bioanalyzer plots showing cfDNA traces and the recovery of a 50 bp ladder fragments from blood plasma collected in standard EDTA blood collection tubes (two independent extractions; blue and red trace respectively, ladder input in green). 2 mL of plasma obtained from EDTA blood collection tubes was spiked with 50 bp DNA ladder (10ng/mL of plasma) and processed using Sera-Xtracta Cell-Free DNA Kit ; 1 µl was ran on High Sensitivity DNA chip alongside 50 bp DNA ladder input.

5.6 Troubleshooting

Please refer the guide for troubleshooting. Should you require further support please contact GE Healthcare Scientific Support. Visit *http://www.gelifesciences.com*for contact information.

cfDNA yield is low

Possible cause	Corrective action
Incorrect handling of blood sample that resulted in degradation of cfDNA prior to purification.	• The kit is intended for cfDNA extraction from blood plasma collected in Streck Cell-Free DNA BCT tubes. Streck stabilization chemistry allows for cfDNA preservation and prevents excessive genomic DNA release. • cfDNA yield depends on the plasma donor and can vary between plasma samples.
Partial loss of materials during the process when opening tubes.	• Make sure the sample volume is pooled as a single bulk volume at the bottom of the tube before opening closed tubes to prevent sample loss.
Partial loss of magnetic beads with bound DNA during the process.	• Make sure the sample volume is pooled as a single bulk volume at the bottom of the tube before magnet settling, as beads in isolated liquid droplets cannot be properly collected and may be lost on aspiration. • Use of weak magnets or inefficient racks may require longer settling times to ensure all beads are collected and so not lost during aspiration.
Binding mix prepared incorrectly, poor pipetting accuracy, incomplete mixing.	• The relative amount of each component in the mix is critical for the maximum cfDNA recovery and minimum binding of genomic DNA; we recommend reverse pipetting to ensure volume accuracy. • Ensure that Binding mix is thoroughly mixed prior to addition to the plasma. • Ensure that plasma/Binding mix solution is thoroughly mixed. • Ensure that the solution is being vigorously mixed during the binding step.

Possible cause	Corrective action
Wash buffer 2 was not completely removed before elution.	• Make sure that the residual traces of Wash buffer 2 are efficiently removed as described in the drying step, before the Elution buffer is added.
Proteinase K activity reduced or lost.	• Proteinase K digestion is essential to reverse the effects of Streck stabilization chemistry. Reconstituted enzyme should be stored at 2°C-8°C and is stable for up to 4 months.

Genomic DNA content is high

Possible cause	Corrective action
Blood sample was degraded before processing for plasma.	• Blood samples should be collected in tubes specifically intended for cfDNA isolation. The kit is validated for Streck Cell-Free DNA BCT tubes. • Do not freeze the blood. • Sera-Xtracta cfDNA kit is designed to reduce the co-purification of higher molecular weight that may be present in plasma. Should significant gDNA contamination occur please ensure correct ratio of Binding mix components is used.
Incorrect quantities of reagents used for cfDNA isolation.	• Make sure volumes of reagents are dispensed as directed.

6 Related products

Item	Product code
Sera-Mag Select	29343045 – 5 mL 29343052 – 60 mL 29343057 – 450 mL
illustra PuReTaq Ready-To-Go PCR Beads	27955701 – multiwell plate, 96 reactions 27955702 – multiwell plate, 5 × 96 reactions 27955801 – 0.5 mL tubes, 100 reactions 27955901 – 0.2 mL hinged tube with cap, 96 reactions
illustra GFX PCR DNA and Gel Band Purification Kit	28903466 – 10 purifications 28903470 – 100 purifications 28903471 – 250 purifications
illustra GFX 96 PCR Purification Kit	28904263 – Mini Spin, 10 purifications 28904264 – 50 purifications 28904265 – 250 purifications
illustra tissue and cells genomicPrep Mini Spin Kit	28904275 – 50 purifications 28904276 – 250 purifications
MagRack Maxi	28986441 for 15 mL / 50 mL tubes
MagRack 6	28948964 for 1.5 mL / 2.0 mL microtubes
Sera-Xtracta Genomic DNA Kit	29429140

7 Quick-Start protocol for manual extraction of 2.0 mL of blood plasma

Required additional equipment and chemicals

- 15 mL centrifuge tubes and 1.5 mL microtubes, ideally DNA Lo-Bind (sterile, DNase-free).
- Mixers e.g., Eppendorf Thermomixers, for 15 mL centrifuge tubes and 1.5 mL microtubes.
- Bench-top centrifuges for 15 mL centrifuge tubes and 1.5 mL microtubes.
- Magnetic separation racks, suitable for 15 mL centrifuge tubes and 1.5 mL microtubes.
- Ethanol (absolute).
- 2-Propanol (isopropanol).

Reagent preparation before first use of kit

- Proteinase K – add DNase-free water to the Proteinase K vial to reconstitute the enzyme at a concentration of 20 mg/mL (e.g. 1.5mL to 30 mg; 3 mL to 60 mg). Mix to dissolve. Store reconstituted reagent at 2°C to 8°C.
- Wash buffer 1 – add ethanol (85 mL) to Wash buffer 1 supplied bottle. Mix thoroughly.
- Wash buffer 2 – add ethanol (140 mL) to Wash buffer 2 supplied bottle. Mix thoroughly.

Input sample

This protocol is validated for blood plasma collected in Streck cell-free DNA BCT tubes and optimized for an input sample of 2.0 mL. For different input volumes and blood collection tubes, see the full IFU online.

Lysis

1. Add 40 uL **proteinase K** solution and 2.0 mL plasma sample into a 15 mL centrifuge tube and mix by brief vortexing.
2. Add 100 µL of **20% SDS** to the sample from step 1.
3. Pulse vortex 2-3 times and mix thoroughly by vortexing for 15 seconds.
4. Incubate at 60°C for 20 minutes.

Binding mix preparation

NOTE: Make sure that the magnetic beads are fully resuspended by vortexing before dispensing.

5. Prepare the **Binding mix** by combining the following (the relative amount of each component in the mix is critical for the maximum cfDNA recovery and minimum binding of genomic DNA; we recommend reverse pipetting to ensure volume accuracy)
    a. 2.9 mL of **Binding buffer** x (no of samples to be processed + 10%)
    b. 1.4 mL of **isopropanol** x (no of samples to be processed + 10%)
    c. 15 µL of Magnetic **Bead suspension** (no of samples + 10%)
6. Mix thoroughly by pulse vortexing.

DNA Binding to magnetic beads

7. Add 4.2 mL of the **Binding mix** to the sample (reverse pipetting recommended).
8. Mix thoroughly by pulse vortexing and incubate in a thermomixer for 10 minutes at 25°C / 1000 rpm.
9. Briefly spin and place the 15 mL tube into a magnet rack for 5 minutes.
10. The beads contain bound cfDNA and once they collect to the magnet carefully aspirate the cleared supernatant to waste.

Bead transfer to 1.5 mL tubes

11. Remove the 15 mL tube from the magnet and add 400 µL of **Wash buffer 1** directly to the bead pellet.
12. Fully resuspend the beads by pulse vortexing, briefly spin.
13. Pipet up and down and transfer the contents of the 15 mL tube to a 1.5 mL microtube (due to the liquid viscosity, expel the content of the tip slowly to ensure complete transfer of the bead suspension).
14. Add a second aliquot of **Wash buffer 1** (400 µL) to the 15 mL tube, vortex, briefly spin and transfer the content to the corresponding 1.5 ml tube from the previous step.
15. Place the 1.5 mL microtube into a magnet rack for 1 minute, discard the supernatant.

Bead Washes

16. Remove the microtube from the magnet, add 700 µL of **Wash buffer 1** and incubate in a thermomixer for 1 minute at 25°C / 1400 rpm.
17. Vortex and briefly spin.
18. Place the microtube into a magnet rack for 1 minute, discard the supernatant.
19. Remove the microtube from the magnet, add 700 µL of **Wash buffer 2** and incubate in a thermomixer for 1 minute at 25°C / 1400 rpm.
20. Vortex and briefly spin.
21. Place the microtube into a magnet rack for 1 minute, discard the supernatant.
22. Repeat steps 19-22.

Drying of beads

23. Briefly centrifuge to collect any residual Wash buffer 2 at the bottom of the tube.
24. Place the microtube back into a magnet rack for 1 minute and carefully remove the cleared residual supernatant from the very bottom of the microtube using a small pipet tip.
25. Allow the bead pellet to air-dry for 5 minutes while on the magnet rack.

Elution of purified cfDNA from the magnetic beads

26. Remove the microtube from the magnet and add 30 µL **Elution buffer** to the sample.
27. Vortex to resuspend the beads and incubate in the thermomixer for 3 minutes at 25°C / 1400 rpm.
28. Briefly spin and place the microtube into a magnet rack for 1 minute.
29. Once the beads collect to the magnet carefully transfer the supernatant containing the isolated cell-free DNA into a fresh microtube. Proceed with analysis.

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GE Healthcare UK Limited, Amersham Place
Little Chalfont, Buckinghamshire, HP7 9NA United Kingdom

www.gelifesciences.com

For local office contact information, visit www.gelifesciences.com/contact

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