Sera-Xtracta™ Cell-Free DNA Kit

EFFICIENT EXTRACTION AND PURIFICATION OF CELL-FREE DNA FROM LIQUID BIOPSY SAMPLES

The Sera-Xtracta™ Cell-Free DNA Kit (Fig 1) is designed for rapid extraction and purification of cell-free DNA (cfDNA) from plasma, serum, and urine. The product selects for small-fragment cfDNA while minimizing any co-purification of higher molecular weight genomic DNA that may be present. The Sera-Xtracta™ Cell-Free Kit performs reliably with commonly used collection tubes including Cell-Free DNA BCT™ collection tubes (Streck™), PAXgene™ Blood ccfDNA Tubes (PreAnalytiX) and Cell-Free DNA Collection Tubes (Roche). The isolation procedure can be completed in two hours to yield high quality cfDNA suitable for downstream applications such as polymerase chain reaction (PCR), digital droplet PCR (ddPCR), genotyping and next generation sequencing (NGS).

With the increasing use of nucleic acid extraction for molecular biology applications, lab managers are focused on increasing nucleic acid purification throughput by using faster, easy to handle, automated procedures that deliver reliable results. The Sera-Xtracta™ Cell-Free DNA Kit facilitates the transition from column-based purification to bead-based purification and provides high-purity extraction with low limits of detection, assay after assay.

Fig 1. The Sera-Xtracta™ Cell-Free DNA Kit includes Proteinase K, SDS 20% aq., magnetic silica bead suspension, binding buffer, two wash buffers and cfDNA Elution buffer for 96 purifications, based on nominal purification input volume of 2.0 mL of plasma.

Cell-free DNA analysis from plasma samples

Plasma was prepared from whole blood (healthy human donors), collected in Cell-Free DNA BCT™ collection tubes. Purifications were carried out using 2 mL plasma eluted with 30 µL of elution buffer. Yield and fragment distribution of cfDNA was analyzed using a High Sensitivity DNA Kit on 2100 Bioanalyzer™ (Agilent™) (Fig 2).

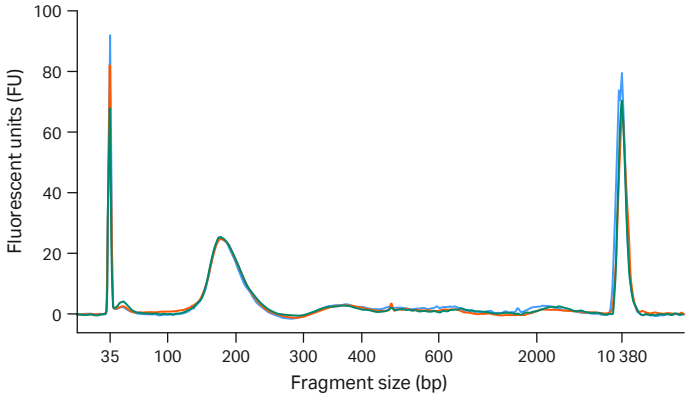


Fig 2. Typical Bioanalyzer traces showing cfDNA profile recovered from a healthy volunteer in three independent experiments (blue, orange, and green trace). Plasma (2 mL) obtained from blood collected in Cell-Free DNA BCT™ collection tubes was processed using Sera-Xtracta™ Cell-Free DNA Kit and 1 µL was run on a High Sensitivity DNA chip on the 2100 Bioanalyzer™.

DNA recovery versus fragment size

Sera-Xtracta™ Cell-Free DNA Kit maximizes the recovery of small degraded cfDNA fragments, reported to be present in plasma of patients with advance stage cancer and to represent a fraction enriched in DNA of tumour origin. At the same time, the kit design considerably reduces any co-purification of higher molecular weight genomic DNA that may be present, originating from lysed blood cells. This is illustrated in Figures 3 and 4 which show the size-dependent recovery of 50 bp ladder fragments.

Compatibility with alternative cfDNA stabilizing tubes

Blood was collected in Cell-Free DNA BCT™ collection tubes, PAXgene™ Blood ccfDNA Tubes and Cell-Free DNA Collection Tubes. Plasma was isolated within six hours post blood draw using a double spin protocol (1600 × g for 10 mins, upper layer transfer, 16 000 × g for 10 mins) and processed using Sera-Xtracta™ Cell-Free DNA Kit. As seen on Figure 3, the recovery of the main cfDNA peak (indicated by an arrow) is indistinguishable between the three blood collection tubes types.

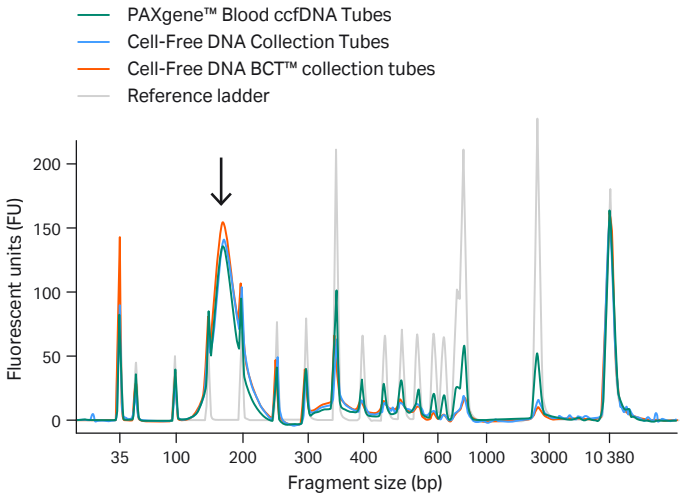


Fig 3. Bioanalyzer plots showing size dependent recovery of a 50 bp ladder fragments used to spike a healthy volunteer plasma collected in Cell-Free DNA BCT™ collection tubes, red trace; PAXgene™ Blood ccfDNA Tubes, green trace; Cell-Free DNA Collection Tubes, blue trace. Reference ladder equivalent to spiked input shown in grey, main cfDNA peak indicated by an arrow.

- Reference ladder equivalent to spiked input shown in grey.
- In all spiked-in samples, ladder fragments corresponding to the main cfDNA peak (i.e. between 100 and 300 bp) and fragments ≤ 100 bp are efficiently recovered.
- Relative recovery of ladder fragments shows minimal recovery at 2.5 kb designed to minimize the recovery of higher molecular weight genomic DNA.

Figure 4 demonstrates the efficient recovery profiles for low molecular weight fragments, i.e. 50 bp and 100 bp, and the minimized recovery profile for high molecular weight fragments of 2.5 kb.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during February and March 2020 and is held at this location. Experiment ID: LS-039181.

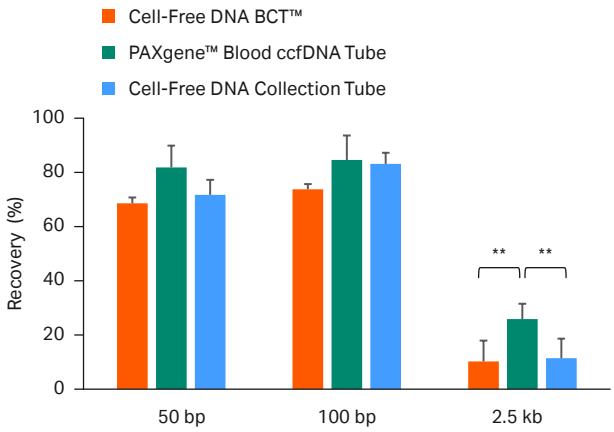


Fig 4. Size dependent recovery of spiked-in DNA ladder for selected fragments from blood plasma collected in the three different cfDNA stabilising tubes as described in the Figure legend. Data reflect the mean obtained from three independent experiments, error bars represent standard deviation, statistical analysis performed using parametric Anova with Tukey's multiple comparisons test.

Scalability

The Sera-Xtracta™ Cell-Free DNA Kit can be used with a range of sample input volumes. Effective purification of cfDNA from plasma input volumes of 0.5 mL to 4 mL has been demonstrated. Elution volumes are scaled in line with input volume, to normalize for concentration (Table 1).

Table 1. Scalable plasma input with corresponding elution volumes to obtain equivalent concentration

Plasma input	**0.5 mL**	**1 mL**	**2 mL**	**4 mL**
Elution volume	15 µL (7.5 µL)	15 µL	30 µL	60 µL

Note: For 0.5 mL plasma input the elution volume may be scaled down accordingly to 7.5 µL; however, due to potential issues with handling volumes below 15 µL it is generally recommended to use 15 µL elution volume.

Purified cfDNA was analyzed using a High Sensitivity DNA Kit on 2100 Bioanalyzer™ (Fig 5).

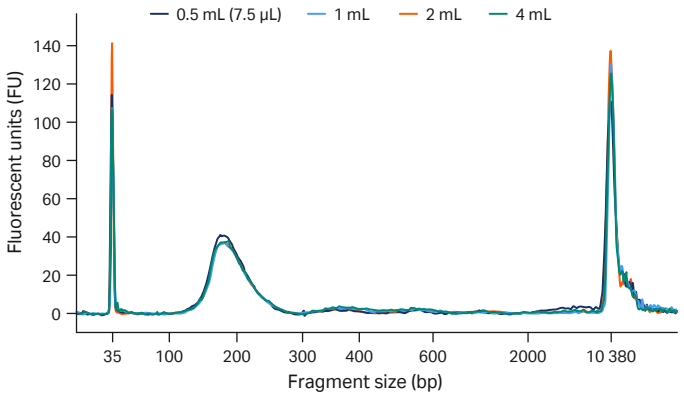


Fig 5. Example Bioanalyzer traces, with 0.5–4 mL plasma input. Samples obtained from blood collected in Cell-Free DNA BCT™ collection tubes.

- Cell-free DNA recoveries are in line with different plasma input volumes.
- Note: For 0.5 mL plasma input the elution volume has been scaled down to 7.5 µL. however due to potential issues with handling volumes below 15 µL it is generally recommended to use 15 µL elution volume.

Comparison of semi-automated and manual processes

Automation of Sera-Xtracta™ Cell-Free DNA Kit on the KingFisher™ Duo Prime (Thermo Fisher Scientific) instrument has also been demonstrated. An automation script* 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 analyzed by 2100 Bioanalyzer™ (Fig 6) and compared to cfDNA isolated with the same isolation kit and sample, using the manual protocol (Fig 7).

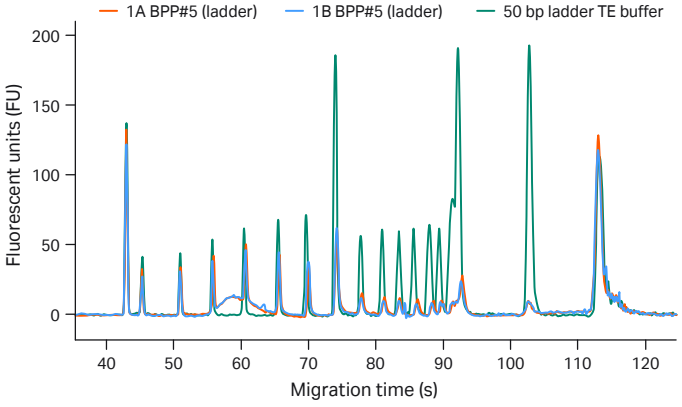


Fig 6. Example Bioanalyzer traces, with 2 mL plasma (obtained from blood collected in Cell-Free DNA BCT™ collection tubes extracted in duplicates on KingFisher™ Duo Prime, spiked with 10 ng/mL of a 50 bp DNA ladder.

- 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.

*An automation script for Kingfisher™ Duo is available on request from Scientific Support (cytiva.com/support/scientific-support)

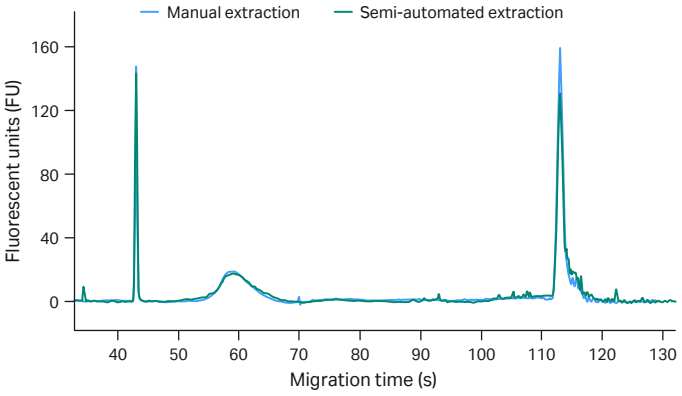


Fig 7. Comparison between manual and semi-automated cfDNA extraction. Plasma (2 mL) obtained from blood collected in Cell-Free DNA BCT™ collection tubes was processed using Sera-Xtracta™ Cell-Free DNA Kit, and 1 µL was run on a High Sensitivity DNA chip on the 2100 Bioanalyzer™.

Functionality in next generation sequencing (NGS)

Cell-free DNA was isolated from plasma obtained from maternal blood collected in Cell-Free DNA BCT™ collection tubes using both Sera-Xtracta™ Cell-Free DNA Kit and QIAamp™ Circulating Nucleic Acid Kit (Qiagen). Isolations were carried out using 2 mL plasma with final elution of 30 µL elution buffer as supplied with respective kits. NGS Libraries were prepared using 25 µL of isolated cfDNA and following quantification, run on NextSeq™ (Illumina) system using low pass genome sequencing to confirm suspected aneuploidy.

Cell-free DNA extracted with both kits allowed for analysis using PrenaTest™ (Eurofins LifeCodexx), for the detection of the fetal aneuploidy of chromosomes 21, 18, 13, X, and Y (Table 2).

Table 2. Comparison of Sera-Xtracta™ Cell-Free DNA Kit with QIAamp™ Circulating Nucleic Acid Kit for detection of fetal aneuploidy using PrenaTest™ shows equivalent performance.

Kit	Collection tubes	Library mean fragment length	Total reads	Mapped reads	Deteced variants	Fetal fraction
QIAamp™ Circulating Nucleic Acid Kit	Cell-Free DNA BCT™ tubes	307	23046585	91.16	Trisomy 21	> 4%
Sera-Xtracta™ Cell-Free DNA Kit	Cell-Free DNA BCT™ tubes	303	20580290	91.35	Trisomy 21	> 4%
QIAamp™ Circulating Nucleic Acid Kit	Cell-Free DNA BCT™ tubes	308	30789670	90.98	Trisomy 18	> 4%
Sera-Xtracta™ Cell-Free DNA Kit	Cell-Free DNA BCT™ tubes	307	17534425	91.27	Trisomy 18	> 4%
QIAamp™ Circulating Nucleic Acid Kit	Cell-Free DNA BCT™ tubes	308	20435487	91.11	diploid	> 4%
Sera-Xtracta™ Cell-Free DNA Kit	Cell-Free DNA BCT™ tubes	303	28699591	91.32	diploid	> 4%

Comparison of Sera-Xtracta™ Cell-Free DNA Kit with MagMAX™ Cell-Free DNA Isolation Kit and QIAamp™ MinElute™ ccfDNA Kit in mutation detection by qPCR

Plasma prepared from the whole blood of a healthy donor (collected in Cell-Free DNA BCT™ collection tubes), was spiked with a 174 bp synthetic DNA fragment containing the EGFR C2573T>G L858R mutation at three different concentrations (Fig 8). Cell-free DNA was extracted from 2 mL of plasma using Sera-Xtracta™ Cell-Free DNA Kit, QIAamp™ MinElute™ ccfDNA Kit (Qiagen), and MagMAX™ Cell-Free DNA Isolation Kit (Thermo Fisher Scientific) in three independent experiments. The amount of the recovered mutant fragment was evaluated in qPCR using TaqMan™ mutation detection assay (Thermo Fisher Scientific). The graph below shows excellent performance of Sera-Xtracta™ Cell-Free DNA Kit in circumstances when mutation is present at a very low level.

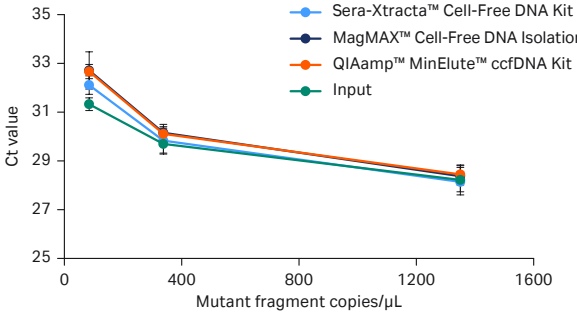


Fig 8. Plasma (2 mL), was spiked with 30 µL of a 174 bp DNA fragment containing the EGFR L858R mutation at 1350, 337.5, and 84.5 copies per µL. The graph shows the average recovery profile of the mutant fragment for each of the three kits. The starting amount of the mutated fragment quantified prior to extraction is shown in green (i.e. input). Error bars represent standard deviation.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocols and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during August and September 2019 and is held at this location.

Comparison of Sera-Xtracta™ Cell-Free DNA Kit with MagMAX™ Cell-Free DNA Isolation Kit and QIAamp™ MinElute™ ccfDNA Kit in the recovery of cfDNA and genomic DNA

Plasma was prepared from whole blood (healthy human donors), collected in Cell-Free DNA BCT™ collection tubes. Purifications were carried out using 2 mL plasma in accordance with kit manufacturer's instructions. Fragment distribution was analyzed using 2100 Bioanalyzer™, with High Sensitivity DNA Kit (Fig 9). The yield of cfDNA and gDNA carry-over was calculated using smear analysis tool (2100 Bioanalyzer™ Expert software, Agilent) within 100–270 bp and 1–9 kb region respectively (Fig 10).

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during August and September 2019 and is held at this location.

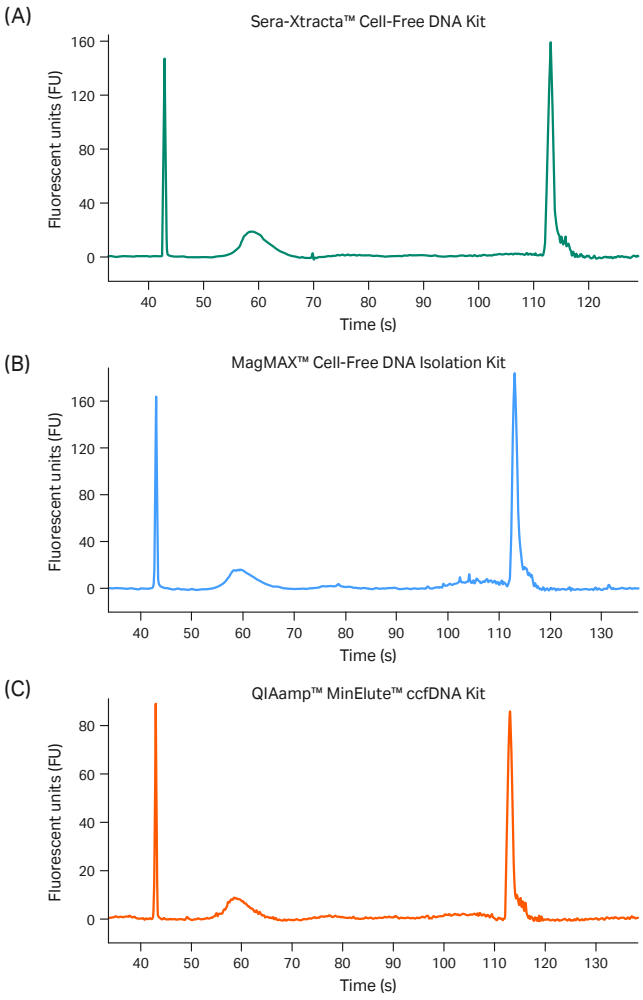


Fig 9. Representative Bioanalyzer traces of cfDNA isolated using (A) Sera-Xtracta™ Cell-Free DNA Kit; (B) MagMAX™ Cell-Free DNA Isolation Kit; (C) QIAamp™ MinElute™ ccfDNA Kit.

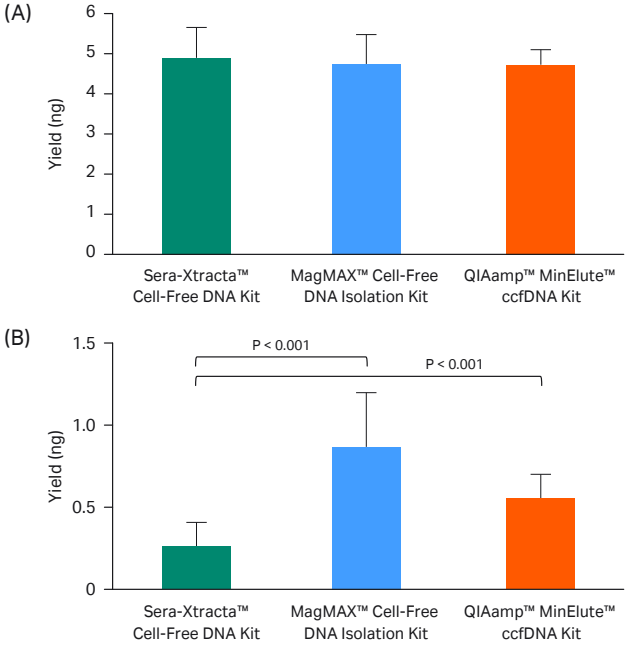


Fig 10. Plotted (A) cfDNA recovery and (B) gDNA carry-over (based on five independent experiments, n=11). Error bars represent standard deviation. Statistical analysis performed using Brown-Forsythe and Welch's ANOVA test with Dunnett's post hoc test. The carryover of high molecular weight genomic DNA (> 1000 bp) is minimal when using Sera-Xtracta™ Cell-Free DNA Kit compared to MagMAX™ Cell-Free DNA Isolation Kit and QIAamp™ MinElute™ ccfDNA Kit.

Results from plasma collected in standard EDTA blood collection tubes

While optimized for isolation of cfDNA from blood collected in Cell-Free DNA BCT™ collection tubes, the Sera-Xtracta™ Cell-Free DNA Kit also performs reliably with blood collected in other types of collection tube. To demonstrate this, plasma (2 mL) obtained from EDTA blood collection tubes was spiked with 50 bp DNA ladder (10 ng/mL of plasma) and processed using Sera-Xtracta™ Cell-Free DNA Kit (Fig 11).

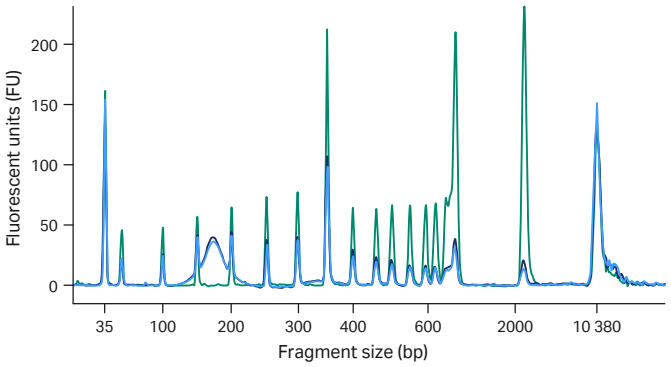


Fig 11. Bioanalyzer plots showing cfDNA traces and the recovery of 50 bp ladder fragments from blood plasma collected in standard EDTA blood collection tubes (two independent extractions; dark and light blue trace respectively, ladder input in green).

Cell-free DNA analysis from serum samples

Sera-Xtracta™ Cell-Free DNA Kit is designed to effectively capture cfDNA from plasma. Due to the reported high level of gDNA in serum samples, we further verified the suitability of the kit for efficient extraction of cfDNA from serum and compared the kit's performance to another bead-based kit: MagMAX™ Cell-Free DNA Isolation Kit. To demonstrate this, 1 mL of serum was used in three independent experiments following manufacturer's instructions. Fragment distribution of the extracted sample was analyzed using capillary electrophoresis on 2100 Bioanalyzer™, with High Sensitivity DNA Kit (Fig 12 A and B). For cfDNA recovery estimation, samples were run on the 7500 DNA chip (Agilent™) and the DNA concentration within main cfDNA peak was calculated with 2100 Bioanalyzer™ Expert software smear analysis tool for fragments between 130–260 bp (Fig 12 C).

In agreement with reported high level of gDNA in serum samples, representative electropherograms in Figure 12 show a significant amount of HMW DNA present in the eluted fraction for MagMAX™ Cell-Free DNA Isolation Kit, while the amount of gDNA carry-over is substantially reduced in the sample processed with the Sera-Xtracta™ Cell-Free DNA Kit. More importantly, the yield of cfDNA is consistently and significantly higher ($p < 0.01$ based on paired t-test) in samples processed with the Cytiva kit (Fig 12), indicating a superior performance of Sera-Xtracta™ Cell-Free DNA Kit with serum samples.

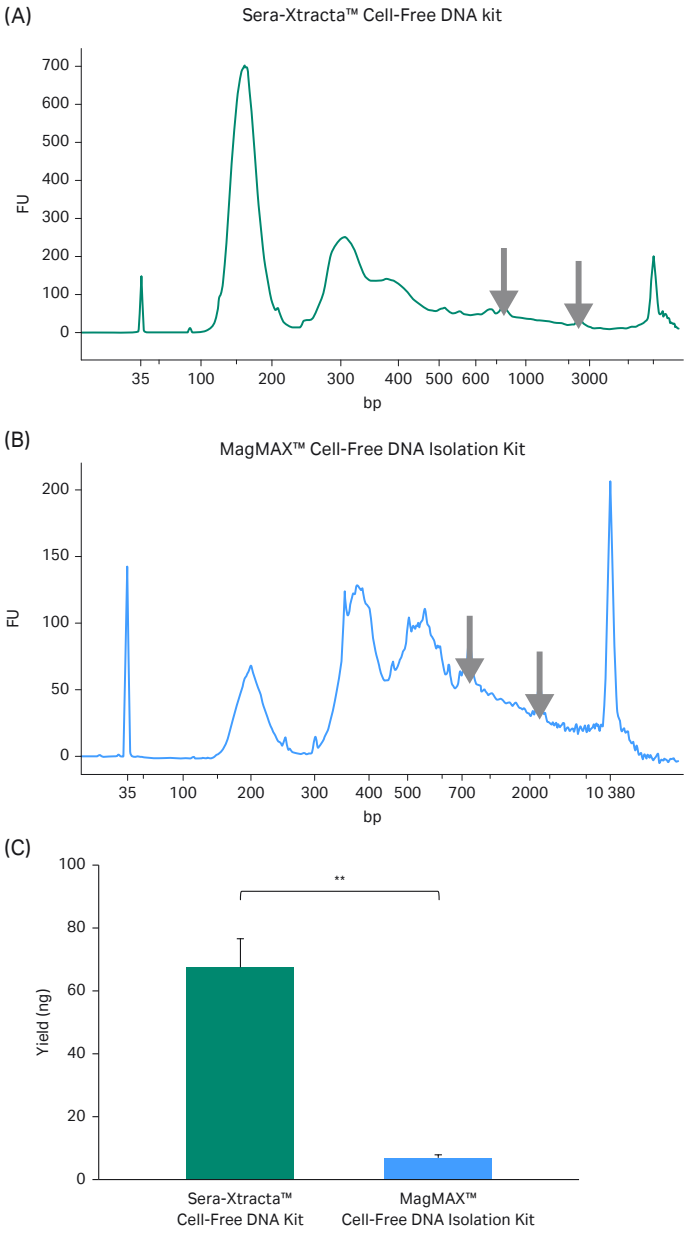


Fig 12. Extraction of cfDNA from serum: representative electropherograms showing DNA fragment distribution obtained from healthy donor serum following cfDNA extraction using A) Sera-Xtracta™ Cell-Free DNA Kit and B) MagMAX™ Cell-Free DNA Isolation Kit and analyzed on 2100 Bioanalyzer™ using High Sensitivity DNA Kit. cfDNA is represented by a main peak centred around 170 bp, presence of HMW DNA represented by arrows. C) Bar chart summarizing cfDNA recovery from serum calculated using smear analysis tool (2100 Expert software) for fragments between 130–260 bp following capillary electrophoresis on 7500 DNA chip. Statistical analysis performed using paired t-test.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during January and February 2020 and is held at this location. Experiment ID LS-039075

Cell-free DNA analysis from patient derived plasma samples collected in standard EDTA and heparin blood collection tubes

To further demonstrate the reliability of the Sera-Xtracta™ Cell-Free DNA Kit when used with blood collected in other types of collection tube, plasma from patient samples collected in both standard EDTA and heparin blood collection tubes was obtained from commercial sources. The cfDNA was isolated using Sera-Xtracta™ Cell-Free DNA Kit, MagMAX™ Cell-Free DNA Isolation Kit, and QIAamp™ MinElute™ ccfDNA Kit as per manufacturers' recommended instructions for further analysis (Fig 13 and 14).

Note: Plasma sample NSCLC170427B contains significant levels of contaminating higher molecular weight genomic DNA that is co-purified along with the cfDNA when using QIAamp™ MinElute™ ccfDNA Kit or MagMAX™ Cell-Free DNA Isolation Kit. However, when using the Sera-Xtracta™ Cell-Free DNA Kit to isolate cfDNA form this sample, this higher molecular weight DNA fraction is greatly reduced.

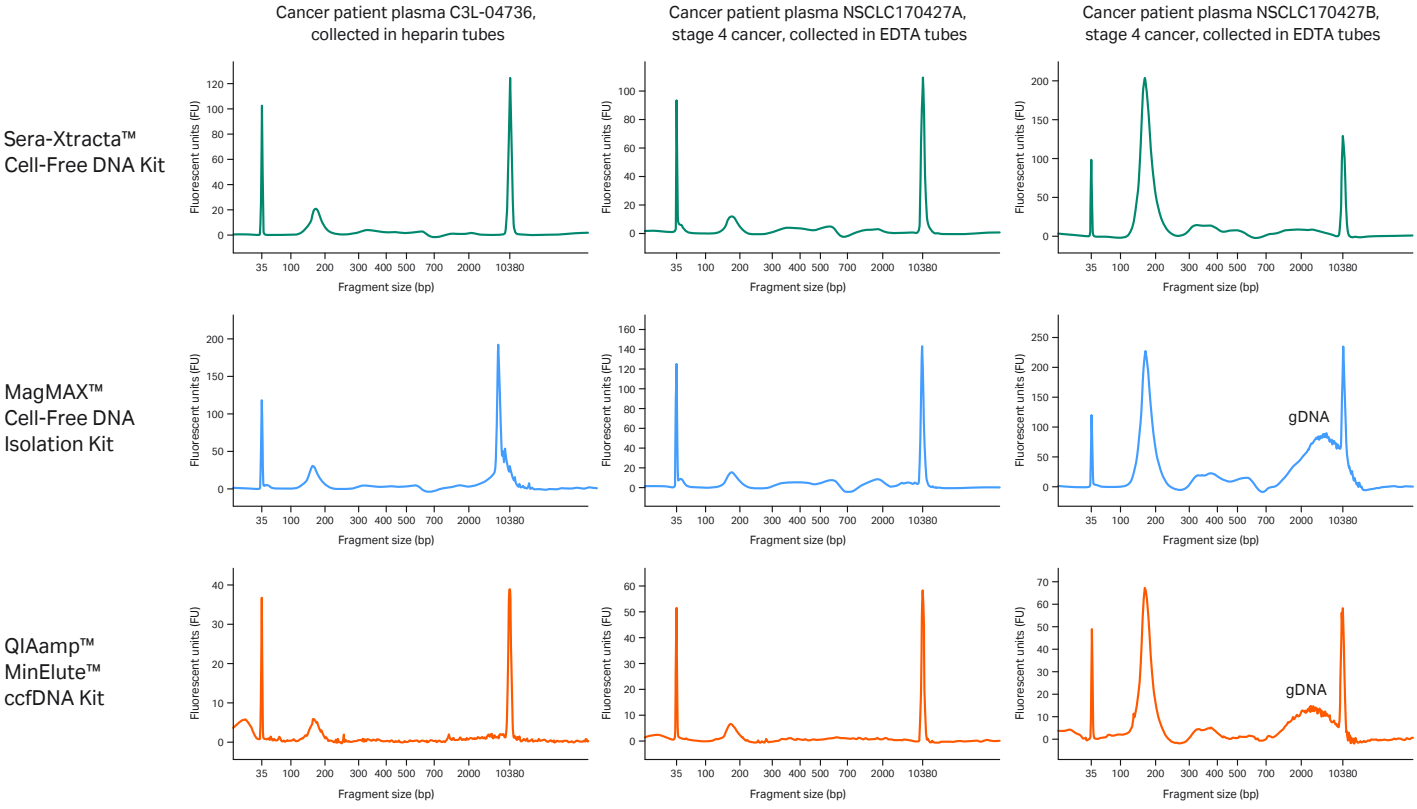
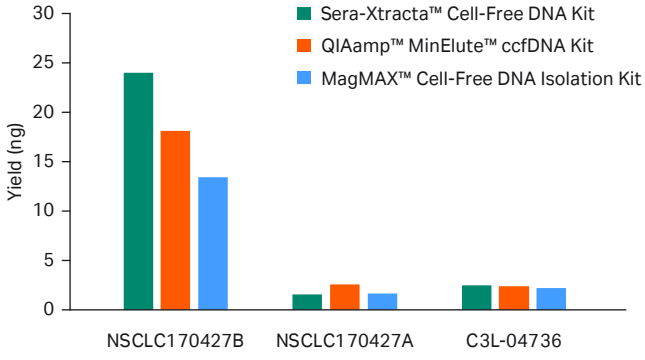


Fig 13. Comparison of isolation performance from both heparin and EDTA blood collection tubes, between Sera-Xtracta™ Cell-Free DNA Kit, MagMAX™ Cell-Free DNA Isolation Kit, and QIAamp™ MinElute™ ccfDNA Kit.



This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during August and September 2019 and is held at this location.

Fig 14. Cell-free DNA recovery (main peak, ~100–300 bp) from patient plasma samples following isolation using Sera-Xtracta™ Cell-Free DNA Kit, MagMAX™ Cell-Free DNA Isolation Kit, and QIAamp™ MinElute™ ccfDNA Kit via 2100 Bioanalyzer™. Scaled for actual recovered extract volumes.

Cell-free DNA analysis from urine samples

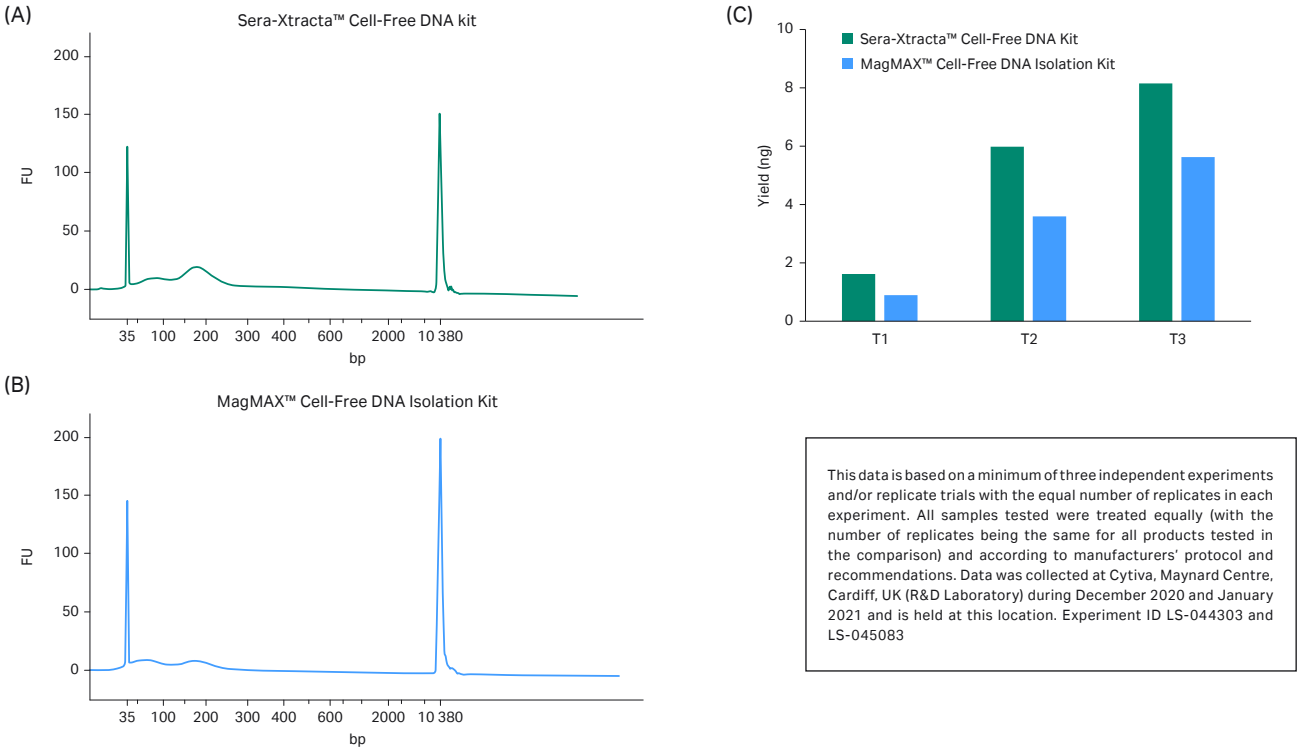
The minimum invasiveness associated with urine collection makes it an attractive alternative to blood for liquid biopsy. Sera-Xtracta™ Cell-Free DNA Kit suitability for efficient extraction of cfDNA from urine samples was evaluated.

The presence of cfDNA in urine is lower than in blood, and is subject to high variation depending on sample collection time points. To address this, urine was collected from a healthy donor at three consecutive collection time points (first void; second void, immediately after exercise; and third void, 1 hour after exercise). The samples were subjected to two consecutive centrifugation steps to remove cellular fraction (750 × g/20 min at 4°C followed by 3000 × g/20 min at 4°C) and 4 mL of urine was processed with Sera-Xtracta™ Cell-Free DNA Kit and MagMAX™ Cell-Free DNA Isolation Kit following manufacturers' protocols (including additional centrifugation step at 16 000 × g).

For cfDNA recovery estimation, samples were separated using capillary electrophoresis on Bioanalyzer with High Sensitivity DNA Kit. Representative electropherograms are presented in Figure 15 A and B. cfDNA yield was calculated with 2100 Expert software smear analysis tool for fragments between 40–300 bp (Fig 15 C).

As presented in Figure 15 the yield of cfDNA is consistently higher ($p < 0.05$ based on paired t-test) in samples processed with Sera-Xtracta™ Cell-Free DNA Kit. These results confirm superior performance of the Cytiva kit in wide range of liquid biopsy samples such as urine and serum.

For direct comparison of the extraction workflow, the Sera-Xtracta™ Cell-Free DNA Kit allows to implement the same extraction method regardless of the input sample, offering exceptional flexibility and faster time to result compared with MagMAX™ Cell-Free DNA Isolation Kit, which requires substantial modification and extension of the standard protocol. This modified method is almost twice as long as the Sera-Xtracta™ Cell-Free DNA procedure (Fig 16).



This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during December 2020 and January 2021 and is held at this location. Experiment ID LS-044303 and LS-045083

Fig 15. Extraction of cfDNA from fresh urine: representative electropherograms showing DNA fragment distribution obtained from a healthy donor urine following cfDNA extraction using A) Sera-Xtracta™ Cell-Free DNA Kit and B) MagMAX™ Cell-Free DNA Isolation Kit and analyzed on 2100 Bioanalyzer™ using High Sensitivity DNA chip C) Bar chart summarizing cfDNA recovery from urine collected at three consecutive time points. cfDNA yield was calculated using smear analysis tool (2100 Expert software) for fragments between 40–300 bp following capillary electrophoresis on 7500 DNA chip. Statistical analysis performed using paired t-test.

Sera-Xtracta™ Cell-Free DNA Kit

MagMAX™ Cell-Free DNA Isolation Kit

Fig 16. Extraction of cfDNA from urine process workflow.

Ordering information

Product	Pack size	Product code
Sera-Xtracta™ Cell-Free DNA Kit	96 purifications*	29437807

*Based on 2 mL sample input of plasma

Related products	Pack size	Product code
Sera-Xtracta™ Genomic DNA Kit	96 purifications (200 µL input)	29429140
Sera-Mag™ Select	5 mL	29343045
	60 mL	29343052
	450 mL	29343057
PuReTaq Ready-To-Go™ PCR Beads	Multiwell plate, 96 reactions	27955701
	Multiwell plate, 5 × 96 reactions	27955702
	0.5 mL tubes, 100 reactions	27955801
	0.2 mL hinged tube with cap, 96 reactions	27955901
GFX™ PCR DNA and Gel Band Purification Kit	10 purifications	28903466
	100 purifications	28903470
	250 purifications	28903471
GFX™ 96 PCR Purification Kit	96 purifications	28903445
MagRack Maxi	15 mL/50 mL tubes	28986441
MagRack 6	1.5 mL/2.0 mL microtubes	28948964

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