Plasma-SeqSensei™

Sample Preparation Guide

Manual guideline

April 2022

SPIFU.R1

en

Glossary of Symbols			
	Manufacturer		Caution
	Consult instructions for use		Keep dry
	Temperature limit		Humidity limit

Table of contents

1 Introduction 3
1.1 Intended purpose 3
1.2 Workflow 3

2 Reagents, consumables and equipment 5
2.1 Non-supplied material 5
2.2 Consumables 5
2.3 Equipment 6

3 Storage and handling 8
3.1 Shipping conditions 8
3.2 General handling precautions 8
3.3 Warnings and precautions 8
3.3.1 Specific measures 9
3.3.2 Handling and storage 9
3.3.3 Reagent handling precautions 10
3.3.4 Safety and contamination precautions 10

4 Procedure 12
4.1 Blood collection and processing 12
4.1.1 Plasma preparation instructions (Whole blood) 12
4.2 Purification of circulating DNA from plasma 14
4.3 Sample quantification (Qubit™) 19

5 Technical assistance 22

6 Glossary and terminologies 23

7 References 24

8 Copyrights and trademarks 25

1 Introduction

Tumour cells undergoing apoptosis, necrosis, or metabolic secretion release small amounts of their DNA into the blood stream. The tumour-specific fraction of circulating free DNA (cfDNA) is called circulating tumour DNA (ctDNA) and contains the genetic information for the primary tumour and even metastases. A multitude of research studies and trials have demonstrated the clinical application of ctDNA profiling at different stages of cancer treatment, including therapy selection, prognosis, and monitoring (1).

Various NGS-based technologies are available for ctDNA detection. However, due to sequencing and PCR bias and errors, most of them are inappropriate for the detection of rare variants. Plasma-SeqSensei™ is a novel NGS-based technology that implements unique molecular identifiers (UID) in the sequencing workflow. This results in a significant error reduction, leading to an ultra-high sensitivity of the PSS technology (2).

To achieve high sensitivity and best results, the preparation of cfDNA from blood samples has to be performed according to the tested procedure described in ► chapter *4 Procedure*, page 12/27.

1.1 Intended purpose

The purpose of this sample preparation guide is to assist users of the PSS *Assay-Specific* Kits with the preparation of cfDNA from blood samples. This guide contains the procedures for plasma purification, cfDNA purification, as well as cfDNA quantification with Qubit™. The workflow starts with blood samples collected in Streck Blood Collection Tubes® for the use in liquid biopsy applications such as Plasma-SeqSense™ *Assay-Specific* Kits and Next-Generation Sequencing (NGS) technologies.

1.2 Workflow

The Plasma-SeqSensei™ workflow is sectioned into multiple steps that must be adhered to Figure 1 describes this process and includes steps for the sample preparation, as well as guidance for specific Instructions for Use (IFUs) to be followed from blood collection to sequencing results.

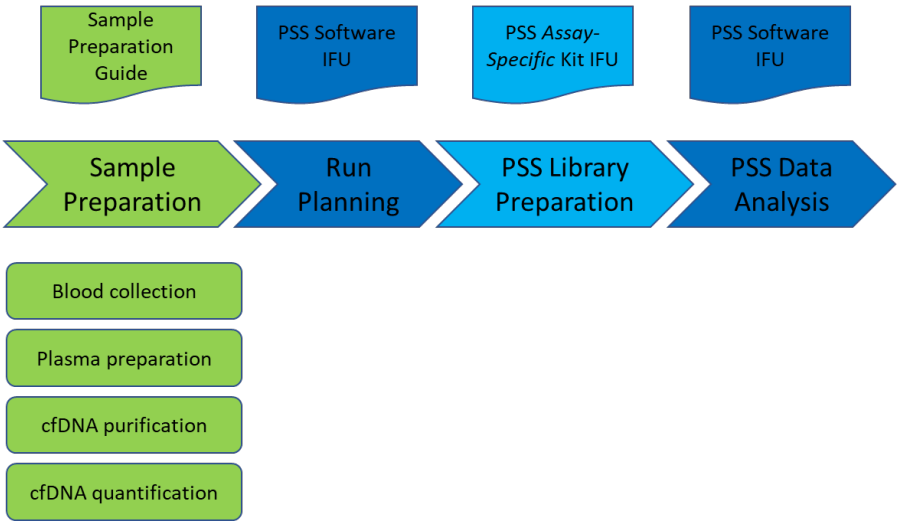

Figure 1: Plasma-SeqSensei™ process, including workflow steps described in Sample Preparation Guide and other required documents.

2 Reagents, consumables and equipment

The workflow described in this guide requires only non-supplied material to prepare blood samples for the use of the Plasma-SeqSensei™ *Assay-Specific* Kits. For the description and use of the Plasma-SeqSensei™ *Assay-Specific* Kit components, please refer to the Plasma-SeqSensei™ *Assay-Specific* Kit IFU (www.sysmex-inostics.com).

2.1 Non-supplied material

Products, where details about manufacturer/vendor and order number are provided in Table 1, Table 2, and Table 3, are essential for the assay and must not be interchanged by products with comparable quality and/or properties.

Table 1: Essential material not provided for Plasma-SeqSensei™ sample preparation

Material	Products
Reagents and kits	* QIAamp® Circulating Nucleic Acid Kit, QIAGEN, #55114
	Ethanol (EtOH) ≥ 99.8 %, p.a.
	2-Propanol ≥ 99.8 %, p.a.
	1X Phosphate buffered saline solution (PBS), (pH 7.4 ± 0.2, without Ca^{2+} and Mg^{2+})
	* Qubit™ 1X dsDNA HS Assay Kit, Thermo Fisher, #Q33230 (100 rxns) or #Q33231 (500 rxns)
	* Qubit™ Assay Tubes, Thermo Fisher, #Q32856

* Essential components; must not be interchanged by products with comparable quality and/or properties.

2.2 Consumables

Table 2: Consumables required for Plasma-SeqSensei™ sample preparation

Laboratory equipment	Product
Blood collection device	* Streck Cell-Free DNA Blood Collection Tubes®, 10.0 ml volume, Streck #218996 for 6 tube pack, Streck #218997 for 100 tube box

Laboratory equipment	Product
Pipette tips/ serological pipettes	Aerosol-resistant sterile pipette tips with filters 10, 200, 1000 µl
	Serological pipette, 5, 10, 25 ml, sterile
Reaction tubes	50, 15, 2, 1.5 ml tubes
	* LoBind® DNA tubes 1,5 ml, Eppendorf, #0030108051
	5 ml cryogenic vial
Safety equipment	Protective glasses
	Protective coats, sleeves, disposable shoe covers, gloves
Miscellaneous	* 3 ml Extension tubes for QIAvac vacuum manifolds, Qiagen, #19587
	* VacConnectors (500) for QIAvac vacuum manifolds, Qiagen, #19407

* Essential components; must not be interchanged by products with comparable quality and/or properties.

2.3 Equipment

Table 3: Equipment required for Plasma-SeqSensei™ sample preparation

Laboratory equipment	Product
Electronic instruments	Centrifuge for 1.5/2 ml tubes, capability of 20,000 × g, fixed angle rotor
	Centrifuge for 15/50 ml tubes, capability of 7,197 × g, fixed angle rotor
	Centrifuge for plasma preparation, capability of 6,000 × g, swing bucket rotor, with low deceleration/acceleration ramp, with adaptors for 15 ml tubes
	Adaptors for fixed-angle rotors for 5 ml cryogenic vials
	Minicentrifuge, capability of ≤ 2,000 × g
	Vortexer with inserts for tubes and 96-well plates
	Thermometer
	Heat block with inserts for 2 ml tubes
	Freezer, -15 °C to -30 °C
	Refrigerator, 2 °C to 8 °C
	Deep freezer, -70 °C to -100 °C

2 Reagents, consumables and equipment

Laboratory equipment	Product
	DNA workstation/PCR cabinet
	Fume hood (strongly recommended)
	Class II Biological Safety Cabinets (strongly recommended)
	* Qiagen Connecting System
	* QIAvac 24 Plus System
	Vacuum pump (230 V, 50 Hz)
	* Qubit™ 3 or 4 Fluorometer
Pipettes	Pipette 1000 µl, 200 µl, 10 µl
	Pipettor 5 to 100 ml
Racks	50 ml, 15 ml, 1.5/2 ml tube rack
	Freezer storage boxes
Miscellaneous	Ice bucket
	Stopwatch

* Essential components; must not be interchanged by products with comparable quality and/or properties.

3 Storage and handling

3.1 Shipping conditions

The preparation of the blood sample for the use of the Plasma-SeqSensei™ *Assay-Specific* Kits requires third-party products that are not supplied. For optimal shipping conditions, follow the manufacturer's instructions for non-supplied material.

Important: Contrary to the manufacturer's instructions, samples collected in Streck cell-free DNA BCT® are to be stored for no longer than 6 days.

3.2 General handling precautions

Ensure that temperature and humidity within the laboratories remain between 15 °C and 30 °C and between 20 % and 85 %, respectively (reduce risk of condensation/evaporation).

Do not eat, drink, or smoke in laboratory areas. Perform equipment maintenance according to manufacturer's instructions.

Decontaminate and dispose of all reagents, specimens, and associated supplies in accordance with government regulations applicable in your location. For accurate and reproducible results, it is essential to avoid contamination with foreign DNA, especially PCR products from previously run samples. The amplified products from previous experiments constitute the most common source of DNA contamination.

3.3 Warnings and precautions

Avoid contact of reagents with the skin, eyes, or mucous membranes. For additional warnings, precautions, and specific measures, refer to the manufacturer's safety data sheets (SDS).

3.3.1 Specific measures

First aid measures:

- ***General advice*:** In case of persisting effects, consult a physician. Remove contaminated clothing and shoes immediately, and launder thoroughly before reusing.
- ***If inhaled:*** Remove the affected person from the immediate area. Ensure supply of fresh air.
- ***In case of skin contact:*** Wash off the affected area with soap and plenty of water.
- ***In case of eye contact***: Remove contact lenses. Rinse the eye thoroughly under running water keeping eyelids wide open for at least 10 to 15 minutes. Protect the unaffected eye.
- ***If swallowed*:** Call a doctor immediately. Do not induce vomiting. Never give anything by mouth to an unconscious person.

3.3.2 Handling and storage

General protective and hygiene measures

Do not eat, drink, or smoke in the laboratory and ensure that good hand washing technique is employed before leaving. Do not inhale vapours. Avoid contact with eyes and skin. Remove soiled or soaked clothing immediately.

Precautions for safe handling

Product handling risks must be minimised by taking the appropriate protective measures and preventative actions. The working process should be designed to rule out the release of the hazardous substances or skin contact as far as it is possible.

Advice on protection against fire and explosion

No special measures necessary.

Conditions for safe storage, including any incompatibilities

Keep the container tightly closed in a dry and well-ventilated place. Opened containers must be carefully resealed and kept upright to prevent leakage.

3.3.3 Reagent handling precautions

To ensure proper use and disposal of reagents and to avoid contamination of reagents, follow the precautions listed below:

- Do not use expired or incorrectly stored reagents.
- Prepare reagents according to manufacturer's instructions.
- Reagents should be used only with reagents from the same kit.
- Reagents from different kits or lots must never be pooled or interchanged.
- Record the open date and mark tubes after each use to ensure reagents are not expired or used beyond the recommended number of freeze-thaw cycles.
- Avoid contamination of reagents by changing gloves frequently. Always change gloves between the handling of reagents and specimens.
- Dispose of unused reagents and waste according to country, federal state, and local environmental regulations.

3.3.4 Safety and contamination precautions

Follow the precautions listed below to maintain a laboratory environment free of DNA contamination and to ensure safety of all personnel:

- Separate the workspaces used for pre- and post-PCR and adhere to a unidirectional workflow from 'clean' (pre-amplification) to 'dirty' (post-amplification) areas.
- Ensure that dedicated equipment (including pipettes), supplies, reagents, biohazard waste containers, and lab manuals are present in each working area. Never exchange these materials between pre- and post-PCR work areas. We recommend colour-coding or labelling of equipment, supplies, and reagents to identify those that belong to a particular area.
- Wear appropriate personal protective equipment throughout the procedure.
    - Wear a lab coat (preferably disposable) and disposable powder-free gloves at all times when working in the pre-PCR and post-PCR areas.

  - To prevent contamination, change gloves frequently between handling specimens and reagents and after contact of the outside of the gloves with the skin.
  - Wear protective eyeglasses during plasma preparation and DNA purification.
  - Wear disposable shoe covers or change shoes between pre- and post-PCR laboratories and wear disposable arm protection sleeves (required in the pre-PCR laboratory).
- When exiting pre- and post-PCR laboratory areas, remove and discard personal protective equipment.
- Handle all specimens as potentially infectious material. If a spill occurs, it is recommended to clean the affected area first with detergent/disinfectant and water and then with ~0.5 % sodium hypochlorite (bleach) solution prepared using deionized water.

  Note: *Commercial liquid household bleach (e.g. Clorox brand) typically contains sodium hypochlorite at a concentration of 5.25 %. A 1:10 dilution of household bleach will produce a 0.5 % sodium hypochlorite solution.*

- Use dedicated PCR cabinets for pipetting steps.
- After use, clean PCR cabinets with quaternary ammonium compounds disinfectant (such as RHEOSEPT-WD plus or equivalent) followed by a product designed for removing nucleic acids and nucleases (such as Roti® Nucleic Acid-Free or equivalent).
- After use, clean PCR workspaces with a product designed for removing nucleic acids and nucleases (such as Roti® Nucleic Acid-Free or equivalent).
- Decontaminate the safety cabinet, PCR workspaces, and labware (pipettes, tube racks, or other equipment) with ultraviolet (UV) light after use. To ensure UV radiation is effective, regularly clean UV bulbs from the accumulating residue.
- Use only aerosol-resistant sterile pipette tips with filters (lot certified, RNase-, DNase-, and pyrogen-free).
- Use only PCR-grade reagents and tubes.
- Keep only one specimen tube or reagent tube open at a time.
- To prevent contamination of multiple-use reagent solutions, prepare working aliquots according to instructions and avoid direct pipetting.

4 Procedure

4.1 Blood collection and processing

The Plasma-SeqSensei™ *Assay-Specific* Kits have been developed using Streck Cell-Free DNA BCT® blood collection tubes. Collect and handle blood according to the manufacturer's instructions. Streck Cell-Free DNA BCT® may be used for samples that are shipped for testing or for in-house laboratory testing.

Ship the specimen at ambient temperature within 24 hours of the blood draw. Avoid temperatures below 6 °C and above 37 °C during shipment.

4.1.1 Plasma preparation instructions (Whole blood)

Start plasma preparation no later than 6 days after the date of the blood draw. Plasma preparation steps must be carried out in quick succession, omitting pauses between individual steps.

Table 4: Required material for plasma preparation from blood

Material	Product
Blood collection device	* Streck Cell-Free DNA Blood Collection Tubes®, 10.0 ml volume, Streck #218996 for 6 tube pack, Streck #218997 for 100 tube box
Pipette tips	Aerosol-resistant sterile pipette tips with filters 200, 1000 µl
Reaction tubes	15 ml tubes
	5 ml cryogenic vial

1. Centrifuge the blood tube at room temperature (between 15 °C and 25 °C) for 10 min at 1,600 x g using a swing-out rotor.

   Note: *To prevent disruption of the cell layer, use a low deceleration ramp of the centrifuge.*

2. After centrifugation, remove tubes carefully from the centrifuge (avoid turbulences).

3. Visually check plasma fraction (supernatant).

   Note: *Soft cell layer may be observed for samples stored at or below 6 °C for a prolonged amount of time (> 12 hours). Haemolysis (light*

pink or reddish plasma) may be observed for samples stored at or above 37 °C for a prolonged amount of time (> 24 hours).

4. Without disturbing the cellular layer, transfer plasma (supernatant) to a fresh 15 ml conical tube by pipetting along the tube wall using a single channel pipette or a disposable bulb pipette. Leave at least 500 µl of residual plasma over the cell layer to avoid its disruption.

   Note: *If soft cell layer is present, transfer only the clear plasma fraction and leave at least 500 µl of residual plasma over the cell layer. If the sample does not show a clear plasma fraction, transfer at least 2.5 ml of plasma from the top.*

   Important: We do not recommend analysis of haemolytic samples due to increased risk of elevated genomic DNA background.

5. Centrifuge the plasma in the 15 ml centrifuge tube at room temperature (between 15 °C and 25 °C) for 10 min at 6,000 x g using a swing-out or fixed angle rotor to remove any residual blood cells.

   Note: *Use a low deceleration ramp of the centrifuge.*

6. After centrifugation, remove tubes carefully from the centrifuge (avoid turbulences).

7. Without disturbing the cell pellet, transfer plasma (supernatant) to a fresh 15 ml centrifuge tube by pipetting along the tube wall using a single channel pipette or disposable bulb pipette. Leave a residual volume of about 300 µl (~7 mm) on the bottom of the tube to avoid contaminating the plasma with cells.

8. Gently mix plasma by pipetting up and down 5 times using a single channel pipette set to 1 ml volume.

9. Transfer plasma into pre-labelled cryogenic vials.

10. If not directly proceeding with ‘Purification of circulating DNA from plasma’ steps, immediately place plasma cryogenic vials in an upright position in a freezer at a temperature between -70 °C and -100 °C for storage (up to a maximum of 24 months).

    Note: *Frozen plasma samples must be shipped on dry ice.*

4.2 Purification of circulating DNA from plasma

For the PSS *Assay-Specific* Kit, the purification of circulating cell-free DNA (cfDNA) from plasma must be performed using the QIAamp® Circulating Nucleic Acid Kit #55114 (QIAGEN). Any volume between 2 and 4 ml plasma per sample may be processed.

Important: Deviating from QIAGEN protocol, do not add carrier RNA during the DNA purification workflow. The DNA elution follows a two-step procedure.

Table 5: Required material for cfDNA purification from plasma

Material	Products
Reagents and kits	* QIAamp® Circulating Nucleic Acid Kit, QIAGEN, #55114
	Ethanol (EtOH) ≥ 99.8 %, p.a.
	2-Propanol ≥ 99.8 %, p.a.
	1X Phosphate buffered saline solution (PBS), (pH 7.4 ± 0.2, without Ca^{2+} and Mg^{2+})
Pipette tips/serological pipettes	Aerosol-resistant sterile pipette tips with filters 20, 200, 1000 µl
	Serological pipette, 5, 10, 25 ml, sterile
Reaction tubes	50, 15, 2, 1.5 ml tubes
	* LoBind® DNA tubes 1.5 ml, Eppendorf, #0030108051
Miscellaneous	* 3 ml Extension tubes for QIAvac vacuum manifolds, Qiagen, #19587
	* VacConnectors (500) for QIAvac vacuum manifolds, Qiagen, #19407

Preparation:

- Turn on the biosafety cabinet and ensure its proper functioning.
- Clean the biosafety cabinet and equipment with DNA-removing agent (e.g. DNA away) as well as disinfecting agent (e.g. 70 % EtOH).
- Set the water bath temperature to 60 °C (±1 °C) and verify the temperature of the water with an external thermometer.
- Set the heat block temperature to 56 °C (±1 °C) and verify the temperature of the blocks with an external thermometer.

- Check the fluid level in liquid waste bottle of the vacuum system. The liquid level must not be above the marked line.
- Check the biohazard waste container. The biohazard solid waste container must not be filled by more than ¾.
- Before starting the work, turn on the vacuum pump to test if it achieves the required vacuum pressure (between -800 and -900 mbar).

When using a new kit, prepare the solutions as described below.

Buffer ACB

- Before use, add 200 ml isopropanol (2-Propanol ≥ 99.8 %, p.a.) to 300 ml Buffer ACB concentrate to obtain 500 ml Buffer ACB. Mix well.

Buffer ACW1

- Before use, add 25 ml EtOH (≥ 99.8 %, p.a.) to 19 ml Buffer ACW1 concentrate to obtain 44 ml Buffer ACW1. Mix well.

Buffer ACW2

- Before use, add 30 ml EtOH (≥ 99.8 %, p.a.) to 13 ml Buffer ACW2 concentrate to obtain 43 ml Buffer ACW2. Mix well.

DNA Purification:

The following steps are performed in the sample preparation area in the pre-PCR laboratory.

Note: *In the following steps, ▲ denotes sample volumes of 2.0-3.0 ml plasma and ● denotes sample volumes of 3.1-4.0 ml plasma.*

1. Allow plasma samples to thaw at room temperature for approximately 15 to 20 min. Do not mix or vortex.

   Note: *The time varies depending on the sample volume and the ambient temperature. Once the samples are thawed, i.e. no more ice is visible in the tube, proceed immediately with the following steps.*

2. When thawed, evaluate the plasma for haemolysis.

Important: We do not recommend analysis of haemolytic samples due to increased risk of elevated genomic DNA background.

3. Prepare all reagents. Fill a collection tube (from the QIAamp® Kit) with ~1 ml of PBS for each sample (to adjust the volume of plasma aliquots).

   Note: *For each sample aliquot, prepare a separate tube with PBS.*

4. Label the screw cap tube and one 50 ml tube per sample.

5. Per test sample: pipette ▲ 300 µl or ● 400 µl QIAGEN proteinase K into a 50 ml tubes and place in the biosafety cabinet.

6. Spin down cryogenic vials containing plasma (brought to a temperature between 15 °C to 25 °C before) at 1,000 x g using a fixed angle rotor. Stop centrifugation once speed is reached.

   Note: *Ensure that the following steps 167 to 10 are performed in a biosafety cabinet in the pre-PCR area.*

7. If centrifuged plasma supernatant volumes are below ▲ 3.0 ml or below ● 4.0 ml, transfer supernatant to a new tube and adjust volumes with PBS to ▲ 3.0 ml or ● 4.0.

8. Add ▲ 3.0 ml or ● 4.0 ml of plasma supernatant per sample to the respective tubes containing proteinase K.

   Note: *When pipetting, avoid disrupting the pellet and leave about 30 µl of plasma at the bottom of the tube.* ***When combining multiple aliquots, open the tubes from only one test subject at a time,*** *i.e. the tubes from which the plasma is to be taken and the tube containing the proteinase K to which the plasma is added.*

9. Add ▲ 2.4 ml or ● 3.2 ml Buffer ACL (without carrier RNA) and close the cap.

10. Mix the tubes by pulse vortexing for 30 s.

    Note: *Pulse vortexing means vortexing in short intervals.*

11. Incubate at 60 °C (±1 °C) in a water bath for 60 min (±2 min).

12. In the meantime, fill the ice bucket with ice or get the cool rack from the -15 °C to -30 °C freezer and label manually the required tubes:

a. One QIAamp® Mini column per sample.

b. 1.5 ml tube per sample.

13. Add ▲ 5.4 ml or ● 7.2 ml Buffer ACB to the lysate in the tube.
14. Close the tube and mix thoroughly by pulse vortexing for 15 s to 30 s.

    Note: *The following steps are independent of the initial sample volume. No further differentiation between 2-3 ml and 3.1-4 ml is indicated.*

15. Incubate the samples on ice or in the cool rack for 5 min (±1 min).
16. Meanwhile, prepare the QIAvac 24 Plus vacuum system:
    - Put the VacConnectors in the slots.
    - Put the labeled QIAamp® Mini columns into the VacConnectors.
    - Put the column extender (extension adapter) on the QIAamp® Mini columns.
17. After incubation on ice, centrifuge the sample tube at 7,000 x g for 30 s to remove the condensate from the lid.
18. With the main vacuum valve still closed, turn on the vacuum pump (set to between -800 and -900 mbar).
19. Carefully transfer the lysate into the column extender (serological pipette). Discard tubes.

    Note: *To prevent cross-contamination, avoid moving the pipette over the column extenders of other QIAamp Mini Columns.*

20. Ensure that the vacuum pressure is in the range between -800 and -900 mbar.
21. Open the main vacuum valve and let the lysate pass through the column completely.
22. Close the main vacuum valve (vacuum stays on) and **maintain vacuum** pressure in QIAvac 24 Plus.
23. Pipette 600 µl Buffer ACW1 into the column extender.

Note: *To avoid cross-contamination, use a fresh pipette tip for each sample and avoid moving pipette tips over the column extender of other samples.*

24. Open the main vacuum valve and let the buffer flow through the column completely.
25. Close the main vacuum valve (vacuum stays on) and **release vacuum** from the QIAvac 24 Plus. Remove the column extender and discard it.

    Note: *To avoid cross-contamination, be careful not to remove the column extenders over other samples/columns.*
26. Apply 750 µl Buffer ACW2 to the QIAamp® Mini column.

    Note: *To avoid cross-contamination, use a fresh pipette tip for each sample and avoid work over the QIAamp® Mini columns containing other samples.*
27. Open the main vacuum valve and let the buffer flow through the column completely.
28. Close the main vacuum valve (vacuum stays on) and **release vacuum** from the QIAvac 24 Plus.
29. Apply 750 µl EtOH (≥ 99.8 %, p.a.) to the QIAamp® Mini column.

    Note: *To avoid cross-contamination, use a fresh pipette tip for each sample and avoid work over the QIAamp® Mini Columns containing other samples.*
30. Open the main vacuum valve and let the EtOH flow through the column completely.
31. Close the main vacuum valve and switch off the vacuum pump.
32. Close the QIAamp® Mini column, remove it from the QIAvac 24 Plus and put it into a 2 ml collection tube. Discard VacConnectors.
33. Centrifugate the samples in Eppendorf Centrifuge 5430 or equivalent at full speed (20,000 x g) for 3 min (±0.5 min).
34. Place the QIAamp® Mini column into a new 2 ml collection tube. Discard the used 2 ml tube.

35. Open the lids and incubate the assembly on a heat block at 56 °C (±1 °C) for 10 min (±1 min) to dry the membrane completely.
36. Place the QIAamp® Mini columns into a clean 1.5 ml elution tubes and discard the 2 ml collection tubes.
37. Carefully apply 70 µl Buffer AVE to the centre of the membrane without touching the membrane.

    Note: *Use a fresh pipette tip for each sample.*
38. Close the lid and incubate at room temperature (between 15 °C and 25 °C) for 3 min (±0.5 min).
39. Centrifuge in Eppendorf Centrifuge 5430 or equivalent at full speed (20,000 x g) for 1 min to elute the nucleic acids.
40. Without touching the membrane, carefully reapply 70 µl Buffer AVE to the centre of the membrane.

    Note: *Use a fresh pipette tip for each sample.*
41. Close the lid and incubate at room temperature (between 15 °C and 25 °C) for 3 min (±0.5 min).
42. Centrifuge in Eppendorf Centrifuge 5430 or equivalent at full speed (20,000 x g) for 1 min to elute the nucleic acids.
43. Discard the QIAamp® Mini column.
44. Store cfDNA in the pre-PCR laboratory at temperatures between 2 °C and 8 °C up to 24 hours or between -15 °C and -30 °C for longer storage times. The purified DNA is stable for at least 1 year.

4.3 Sample quantification (Qubit™)

The Qubit™ Assay is used to quantify cfDNA amounts extracted from plasma samples. DNA amounts are reported in ng/µl.

Note: *Qubit measurement of the samples merely represents a rough approximation of input DNA to determine the sample load. The final and possibly differing quantification of the samples will occur during the sequencing of the library using the internal quantifier (Quantispike).*

The PSS *Assay-Specific* Kits have been developed with Qubit™ 1X dsDNA HS Assay (assay range: 10 pg/µl – 100 ng/µl).

Table 6: Required material for sample quantification using Qubit™

Material	Products
Reagents and kits	* Qubit™ 1X dsDNA HS Assay Kit, Thermo Fisher, #Q33230 (100 rxns) or #Q33231 (500 rxns)
	* Qubit™ Assay Tubes, Thermo Fisher, #Q32856
Pipette tips	Aerosol-resistant sterile pipette tips with filters 2, 10, 20, 200 µl

The following steps are performed in the sample preparation area in the pre-PCR laboratory.

Qubit™ Measurement:

Perform protocol as described in the manual provided by the manufacturer using 5 µl of sample (see Table 7).

Table 7: Determination of DNA concentration with Qubit™

	DNA input	Qubit™ 1X dsDNA HS working solution (Component A)
Standard	10 µl	190 µl
Sample	5 µl	195 µl

Calculate the **Total DNA input**:

$$\boldsymbol{DNA\ input\ per\ sample} = measured\ concentration\ in\ ng/\mu l * 116\ \mu l$$

$$\boldsymbol{Total\ DNA\ input} = \sum measured\ concentrations\ of\ all\ samples$$

Note: *Each **positive control contains 4.3 ng of DNA**, which needs to be considered in the total DNA input for each sequencing run. The positive control DNA will be automatically included in the calculation by the run planning module of the PSS Software*.

Purified cfDNA can be stored in the pre-PCR laboratory at temperatures between 2 °C and 8 °C for up to 24 hours or between -15 °C and -30 °C for longer storage times. The purified DNA is stable for at least 1 year.

Next Steps:

Refer to the PSS Software IFU for run planning and the PSS *Assay-Specific* Kit IFU for the preparation of the sequencing library (see Figure 1).

5 Technical assistance

If problems occur during sample preparation, please refer to the manufacturer's troubleshooting guide if available or contact the manufacturer of the product for assistance.

6 Glossary and terminologies

Term	Definition
BCT	Blood Collection Tubes
cfDNA	Cell-free DNA
ctDNA	Circulating tumour DNA
DNA	Desoxyribonucleic acid
EtOH	Ethanol
IFU	Instructions for use
NGS	Next-generation sequencing
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PSS	Plasma-SeqSensei™
RNA	Ribonucleic acid
SDS	Safety data sheet
UID	Unique identifier
UV	Ultraviolet

7 References

1) Stewart CM, Kothari PD, Mouliere F, Mair R, Somnay S, Benayed R, Zehir A, Weigelt B, Dawson SJ, Arcila ME, Berger MF, Tsui DW. The value of cell-free DNA for molecular pathology. J Pathol. 2018 Apr;244(5):616-627. doi: 10.1002/path.5048. Epub 2018 Mar 12. PMID: 29380875; PMCID: PMC6656375.

2) Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A. 2011 Jun 7;108(23):9530-5. doi: 10.1073/pnas.1105422108. Epub 2011 May 17. PMID: 21586637; PMCID: PMC3111315.

8 Copyrights and trademarks

Plasma-SeqSensei™ is a trademark of Sysmex Corporation, Japan.

Sysmex Inostics GmbH
Falkenried 88
20251 Hamburg, Germany
www.sysmex-inostics.com

April 2022
SPIFU.R1