September 2016

QIAseq™ cfDNA All-in-One T Kit Handbook

For combined cfDNA isolation and library preparation for next-generation sequencing (NGS) applications using Ion Torrent™ instruments

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAseq cfDNA All-in-One T Kit		
Catalog no.		**180043**
Number of reactions		**24**
Component	**Tube color**	**Quantity**
QIAGEN® Mini columns		24
Tube Extenders (20 ml)		1 x 25
Collection Tubes (2.0 ml)		1 x 50
Elution Tubes (1.5 ml)		1 x 50
VacConnectors		1 x 50
Buffer ACL*		1 x 110 ml
Buffer ACB* (concentrate)		3 x 60 ml
Buffer ACW1* (concentrate)		1 x 19 ml
Buffer ACW2† (concentrate)		1 x 13 ml
Buffer AVE†	Violet	2 x 2 ml
QIAGEN Proteinase K		2 x 7 ml
All-in-One Enzyme Mix	Violet	1 tube
All-in-One Reaction Buffer, 4x	Blue	1 tube
HiFi PCR Master Mix, 2x	Green	2 tubes
Primer Mix Ion Torrent Library Amp	Clear	1 tube
RNase-Free Water (1.9 ml)	Clear	1 tube
Adapter Plate 24-plex Ion Torrent		1 plate
Quick-Start Protocol		1

* Contains chaotropic salt. See page 5 for safety information.

† Contains sodium azide as a preservative.

Shipping and Storage

The QIAseq cfDNA All-in-One T Kit comes in two boxes. The QIAseq cfDNA Library T Kit is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. The QIAseq cfDNA Extraction Kit is shipped and stored at ambient temperature. QIAamp® Mini columns should be stored at 4°C upon receipt.

Intended Use

The QIAseq cfDNA All-in-One T Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in a convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solution directly to waste containing Buffer ACL, Buffer ACB or Buffer ACW1.

Buffer ACL, Buffer ACB and Buffer ACW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then clean with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAseq cfDNA All-in-One T Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

Free-circulating nucleic acids, such as tumor-specific extracellular DNA fragments in blood or fetal nucleic acids in maternal blood, are present in plasma usually as short fragments of <500 bp (DNA). The concentration of free-circulating DNA (cfDNA) in plasma is usually low and can range considerably from 1–100 ng/ml among different individuals.

Next-generation sequencing (NGS) is a driving force for numerous new and exciting applications, including cancer research and non-invasive prenatal testing (NIPT). While NGS technology is continuously improving, library preparation particularly from cfDNA, remains a process bottleneck for many labs.

The QIAseq cfDNA All-in-One T Kit combines reliable sample extraction with efficient library prep to maximize success in NGS analysis from plasma samples.

QIAseq cfDNA Extraction Kits enable efficient purification of cfDNA from human plasma. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once). Extension tubes and vacuum processing on the QIAvac 24 Plus allow use of starting sample volumes of up to 5 ml, and flexible elution volumes between 20 µl and 100 µl enable concentration of nucleic acid species that are present in low amounts in the sample material. Free-circulating cell-free DNA is eluted in Buffer AVE, ready for use in subsequent library preparation, as well as amplification reactions or storage at –30°C to –15°C. Purified nucleic acids are free of proteins, nucleases and other impurities.

The QIAseq cfDNA Library T Kit is designed to be the definitive solution for generating high-quality libraries from free-circulating DNA samples that are compatible with all Ion Torrent sequencers. Intended for NGS researchers who seek efficient library preparation to enable new insights, the kit delivers NGS libraries from extracted cfDNA in less than 2.5 hours, maximizing performance for any NGS-based liquid biopsy.

Principle and Procedure

QIAseq cfDNA Extraction Kit

The QIAseq cfDNA extraction procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using QIAamp Mini columns on a vacuum manifold. The robust procedure helps to eliminate sample-to-sample cross-contamination, which is particularly important for NGS applications, and increases user safety when handling potentially infectious samples. The simple procedure, which is highly suited for simultaneous processing of multiple samples, provides pure nucleic acids in less than 2 hours for 24 samples.

Sample volumes

QIAamp Mini columns can bind fragmented nucleic acids that are as short as 20 bases, but the yield depends on the sample volume and the concentration of circulating nucleic acids in the sample. The QIAseq cfDNA Extraction procedure has been optimized for large sample volumes of up to 5 ml.

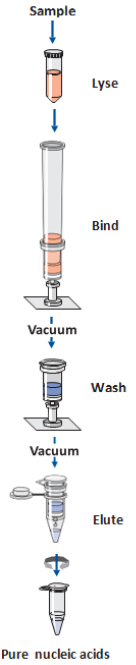


Figure 1. Column-based, manual, cell-free DNA extraction procedure.

Lysing samples

Free-circulating nucleic acids in biological fluids are usually bound to proteins or enveloped in vesicles, requiring an efficient lysis step in order to release nucleic acids for selective binding to the QIAamp Mini column. Hence, samples are lysed under highly denaturing conditions at elevated temperatures in the presence of Proteinase K and Buffer ACL, which

together ensure inactivation of DNases and RNases and complete release of nucleic acids from bound proteins, lipids and vesicles.

Adsorption to the QIAamp Mini membrane

Binding conditions are adjusted by adding Buffer ACB to allow optimal binding of the circulating nucleic acids to the silica membrane. Lysates are then transferred onto a QIAamp Mini column, and circulating nucleic acids are adsorbed from a large volume onto the small silica membrane as the lysate is drawn through by vacuum pressure. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit enzymatic reactions within the library preparation procedure, are not retained on the QIAamp Mini membrane. A vacuum manifold (e.g., the QIAvac 24 Plus with the QIAvac Connecting System) and a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN Vacuum Pump) are required for the protocol. A vacuum regulator should be used for easy monitoring of vacuum pressures and convenient vacuum release.

Removal of residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away during three wash steps. In a single step, highly pure circulating nucleic acids are eluted in Buffer AVE and equilibrated to room temperature.

Elution of pure nucleic acids

Elution is performed using Buffer AVE. The elution volume can be as low as 40 µl. The eluate volume recovered can be up to 5 µl less than the volume of elution buffer applied to the column; for example, an elution volume of 50 µl results in >45 µl final eluate. Eluted cfDNA is collected in 1.5 ml microcentrifuge tubes (provided). If the purified cfDNA is to be stored for up to 24 hours, storage at 2–8°C is recommended. For periods of storage longer than 24 hours, storage at –30°C to –15°C is recommended.

QIAseq cfDNA Library T Kit

Starting from very clean, naturally fragmented double-stranded cfDNA, the QIAseq cfDNA Library T Kit for Ion Torrent sequencers uses a proprietary All-in-One reaction that combines end-polishing and adapter ligation in a single step. Together with QIAGEN's proprietary HiFi PCR Master Mix, this combination maximizes the conversion rate of sample cfDNA into NGS library, while efficiently and evenly amplifying even high and low G/C content regions of the genome. Starting from 1–100ng cfDNA input, this protocol delivers the highest possible yield of a sequencing library that is free of adapter dimer contamination. Due to the kit's flexible protocol, the same kit can also be used for higher DNA input amounts.

Barcoded sequencing adapter plates

Equimolar mixes of barcode and universal adapters, pre-added to single-use microtiter plates, are included with the QIAseq cfDNA Library T Kit (24) for Ion Torrent sequencers. In the 24-plex adapter plate, only the first three columns contain mixes of universal and barcode adapters. The QIAseq cfDNA All-in-One T Kit supports up to 24-plex pooling of libraries prior to sequencing (see Appendix B for adapter barcode sequencing information).

Bead-based cleanup

The QIAseq cfDNA Library T Kit, with its innovative buffer and enzyme formulations, provides an optimized solution to efficiently construct libraries for Ion Torrent sequencers from as little as 1 ng input DNA. Following adapter ligation and library amplification steps, reaction cleanup and removal of residual adapter dimers can be achieved by using Agencourt® AMPure® XP beads, which enable easy automation on various high-throughput automation platforms.

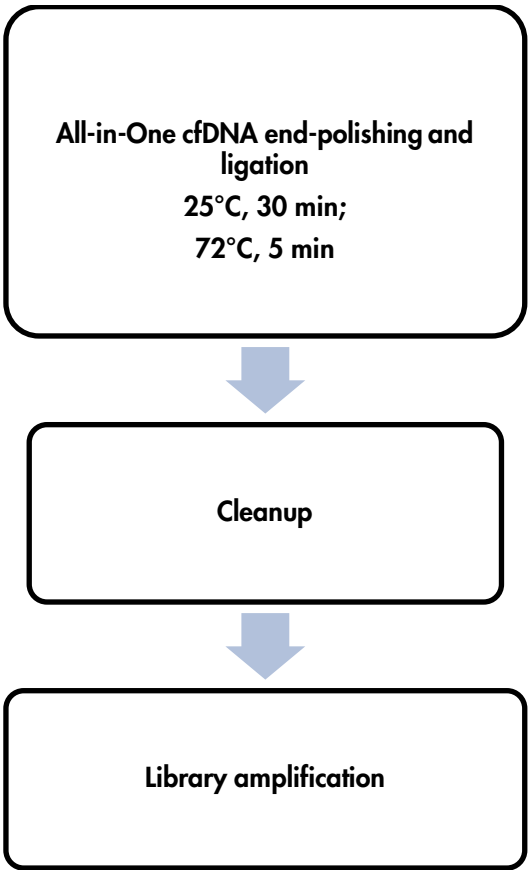


Figure 2. QIAseq cfDNA Library preparation procedure.

Automation

Automated QIAseq sample preparation on the QIAcube®

Purification of cfDNA using the QIAseq cfDNA Extraction Kit can be semi-automated on the QIAcube. The lysis and vacuum steps are performed prior to use of the QIAcube. Due to the high sample input volume of up to 5 ml, only the three wash steps and the elution step can be automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., wash, bind and elute), allowing you to continue using the QIAseq cfDNA Extraction Kit for purification of high-quality cfDNA. For more information about the automated procedure, see the relevant protocol sheet on cfDNA extraction using QIAamp Mini Columns available at **www.qiagen.com/MyQIAcube**.

PCR setup on the QIAgility®

The QIAgility provides rapid, high-precision PCR setup in almost all plate and tube formats and can therefore be used for PCR setup of the library enrichment PCR or qPCR setup for the quality assessment of generated libraries. The easy-to-use software is plug-and-play and requires no special programming, and optimized protocols assure fast startup and immediate results.

Automated library construction

Due to the highly streamlined, one-tube protocol, the automation friendly, single-use barcode adapter plates and magnetic-beads-based size selection and purification, QIAseq cfDNA Library Preparation protocols can be easily automated on commonly used liquid-handling platforms.

Description of protocols

Sample preparation

Two sample preparation protocols are provided in this handbook for different volumes of plasma: one protocol for ≤3 ml plasma and one for processing 4–5 ml plasma.

Library preparation

The library preparation described in this handbook contains two subsequent protocols for generation of cfDNA libraries that are for use on NGS platforms from Ion Torrent. The first protocol describes end-polishing, adapter ligation, cleanup and size selection of cfDNA – to generate libraries for next-generation sequencing. The second protocol describes an optional, high-fidelity amplification step that can be used to ensure high amounts of cfDNA library from as little as 1 ng of starting material.

Starting materials

Plasma samples are used for the combined sample and library prep workflow.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

For sample preparation

- Sterile pipet tips (pipet tips with aerosol barriers are recommended to help prevent cross-contamination) and pipets
- Water bath or heating block capable of holding 50 ml centrifuge tubes at 60°C
- Heating block or similar at 56°C (capable of holding 2 ml collection tubes)
- Microcentrifuge
- 50 ml centrifuge tubes
- QIAvac 24 Plus vacuum manifold (cat. no. 19413)
- QIAvac Connecting System (cat. no. 19419) or equivalent
- Vacuum Pump (cat. no. 84010 [USA and Canada], 84000 [Japan] or 84020 [rest of world]) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- Ethanol (96–100%)*
- Isopropanol (100%)
- Crushed ice
- Some samples may require dilution with phosphate-buffered saline (PBS)
- Optional: VacValves (cat. no. 19408)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethyl ketone.

For library preparation

- Sterile pipet tips and pipets
- Agencourt AMPure XP beads (Beckman Coulter Inc., cat. no. A63880, A63881) for bead-based library purification
- 100% ethanol (ACS grade)
- Nuclease-free water
- Buffer EB (QIAGEN, cat. no. 19086)
- PCR tubes or plates
- Vortexer
- Microcentrifuge
- Thermocycler
- Magnetic stand
- Capillary electrophoresis device (e.g., QIAGEN's QIAxcel® Advanced, Agilent® Bioanalyzer or similar method to assess the quality of DNA library)
- Rotor-Gene® Q qPCR instrument and QIAseq Library Quant Array Kit (QIAGEN, cat. no. 333304) or a similar real-time PCR cycler and method for qPCR-based library quantification

Important Notes

Preparation of buffers and reagents

Buffer ACB

Before use, add 200 ml isopropanol (100%) to 300 ml Buffer ACB concentrate to obtain 500 ml Buffer ACB. Mix well after adding isopropanol.

Buffer ACW1

Before use, add 25 ml ethanol (96–100%) to 19 ml Buffer ACW1 concentrate to obtain 44 ml Buffer ACW1. Mix well after adding ethanol.

Buffer ACW2

Before use, add 30 ml ethanol (96–100%) to 13 ml Buffer ACW2 concentrate to obtain 43 ml Buffer ACW2. Mix well after adding ethanol.

The QIAvac 24 Plus

The QIAvac 24 Plus is designed for fast and efficient vacuum processing of up to 24 QIAGEN spin columns in parallel. Samples and wash solutions are drawn through the column membranes by vacuum instead of centrifugation, providing greater speed and reduced hands-on time in purification procedures.

In combination with the QIAvac Connecting System, the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through is collected in a separate waste bottle.

For maintenance of the QIAvac 24 Plus, refer to the handling guidelines in the *QIAvac 24 Plus Handbook*.

Processing QIAamp Mini columns on the QIAvac 24 Plus

QIAamp Mini columns are processed on the QIAvac 24 Plus using disposable VacConnectors and reusable VacValves. VacValves (optional) are inserted directly into the luer slots of the QIAvac 24 Plus manifold and ensure a steady flow rate, facilitating parallel processing of samples of different natures (e.g., blood and body fluids), volumes or viscosities. They should be used if sample flow rates differ significantly in order to ensure consistent vacuum. VacConnectors are disposable connectors that fit between QIAamp Mini columns and VacValves or between the QIAamp Mini columns and the luer slots of the QIAvac 24 Plus. They prevent direct contact between the spin column and VacValve during purification, thereby avoiding any cross-contamination between samples. VacConnectors are discarded after a single use. Due to the large solution volumes used, the QIAvac Connecting System (or similar setup with waste bottles) is required (see Figure 3).

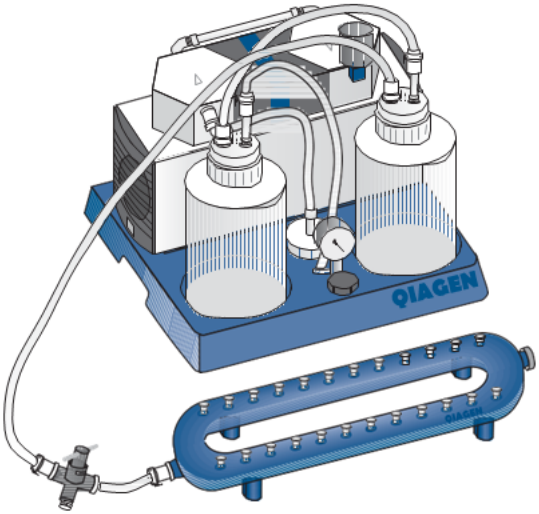


Figure 3. QIAVac 24 Plus, QIAvac Connecting System and Vacuum Pump.

Handling guidelines for the QIAvac 24 Plus

- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus manifold may crack.
- Always store the QIAvac 24 Plus clean and dry. For cleaning procedures, see the *QIAvac 24 Plus Handbook*.
- The components of the QIAvac 24 Plus are not resistant to certain solvents (Table 1). If these solvents are spilt on the unit, rinse unit thoroughly with water.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus manifold.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.
- The vacuum pressure is the pressure differential between the inside of the vacuum manifold and the atmosphere (standard atmospheric pressure 1013 millibar or 760 mm Hg) and can be measured using the QIAvac Connecting System or a vacuum regulator. The protocols require a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN, Vacuum Pump). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce nucleic acid yield and purity and increase the risk of clogged membranes.

Table 1. Chemical resistance properties of QIAvac 24 Plus

Resistant to		
Acetic acid	Chaotropic salts	Chlorine bleach
Chromic acid	Concentrated alcohols	Hydrochloric acid
SDS	Sodium chloride	Sodium hydroxide
Tween® 20	Urea	
Not resistant to		
Benzene	Chloroform	Ethers
Phenol	Toluene	

Setup of the QIAvac 24 Plus vacuum manifold

1. Connect the QIAvac 24 Plus to a vacuum source. If using the QIAvac Connecting System, connect the system to the manifold and vacuum source as described in Appendix A of the *QIAvac 24 Plus Handbook*.
2. Insert a VacValve (optional) into each luer slot of the QIAvac 24 Plus that is to be used (see Figure 4). Close unused luer slots with luer plugs or close the inserted VacValve.
3. VacValves should be used if flow rates of samples differ significantly to ensure consistent vaccum.
4. Insert a VacConnector into each VacValve (see Figure 3).
5. Perform this step directly before starting the purification to avoid exposure of VacConnectors to potential contaminants in the air.
6. Place the QIAamp Mini columns into the VacConnectors on the manifold (see Figure 4).

   Note: Save the collection tube from the plastic bag for use in the purification protocol.
7. Insert a tube extender (20 ml) into each QIAamp Mini column (see Figure 4).

   Note: Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.
8. For nucleic acid purification, follow the instructions in the protocols. Discard the VacConnectors appropriately after use.
9. Leave the lid of the QIAamp Mini column open while applying vacuum.

10. Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during processing. For faster vacuum release, a vacuum regulator should be used.

    Note: Each VacValve can be closed individually when the sample is completely drawn through the spin column, allowing parallel processing of samples of different volumes or viscosities.

11. After processing samples, clean the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).

    Note: Buffers ACL, ACB and ACW1 are not compatible with disinfecting agents containing bleach. See page 5 for safety information.

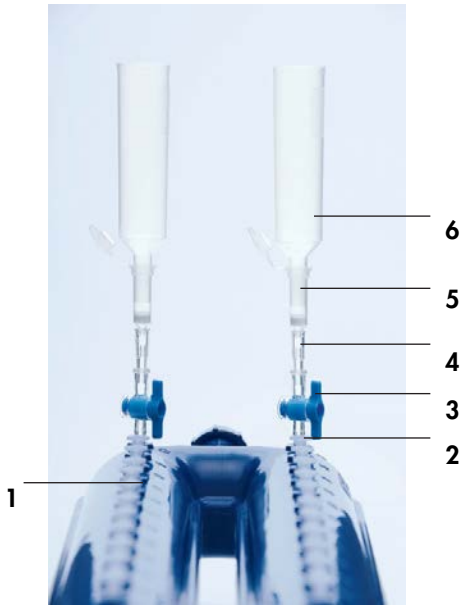


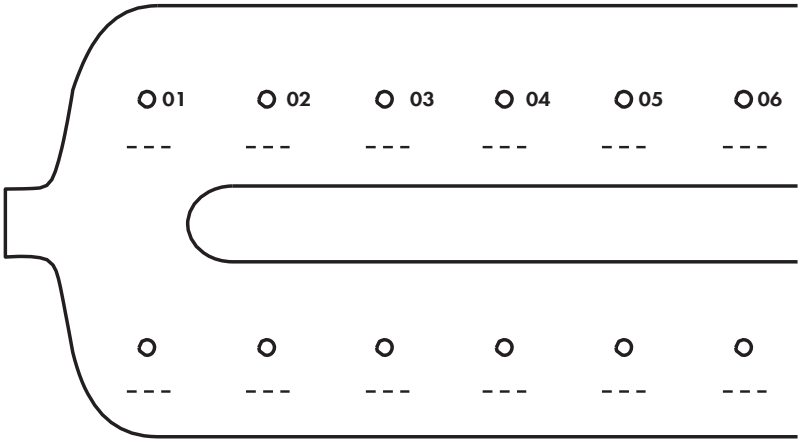
Figure 4. Setting up the QIAvac 24 Plus with QIAamp Mini columns using VacValves, VacConnectors and Tube Extenders. (1: QIAvac 24 Plus vacuum manifold, 2: Luer slot of the QIAvac 24 Plus (closed with luer plug), 3: QIAamp Mini column, 4: VacConnector, VacValve (must be purchased separately), 6: Tube Extender).

We recommend labeling the tubes and the QIAamp Mini columns for use on the QIAvac 24 Plus vacuum system according to the scheme in Figure 5 in order to avoid the mixup of samples. This figure can be photocopied and labeled with the names of the samples.

Date: _____

Operator: _____

RunID: _____



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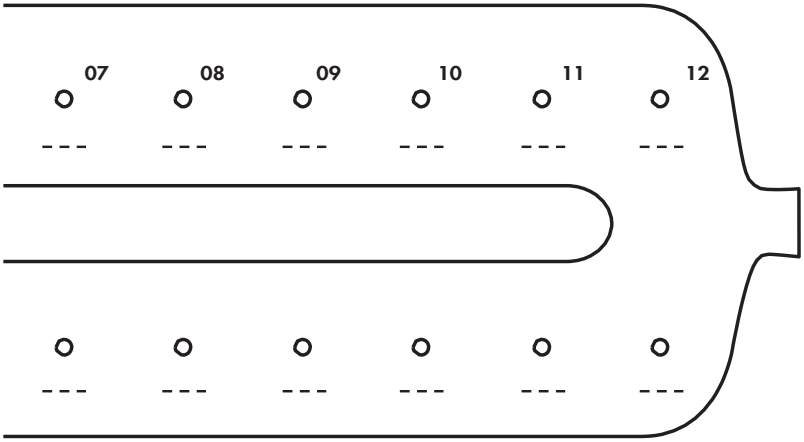


Figure 5. Labeling scheme for tubes and QIAamp Mini columns for use on the QIAvac 24 Plus vacuum system.

Recommended library quantification method

We recommend final library quantification by qPCR using primers complementary to the platform-specific adapters. This allows measurement of only complete library molecules, which are the only molecules able to perform emulsion PCR on Ion Torrent instruments. QIAGEN's QIAseq Library Quant Array Kit (cat. no. 333304) is highly recommended for accurate qPCR quantification of the prepared library. The QIAseq Library Quant Array Kit is compatible with all major NGS platforms and qPCR instruments, and includes pre-dispensed, sequentially diluted DNA standard to eliminate manual titration steps.

For the most accurate qPCR analysis using the QIAseq Library Quant Array Kit Excel® sheet, the library fragment size distribution should be as narrow as possible, with a known mean value in base pairs. Wide fragment distributions are more difficult to accurately quantitate by qPCR, and the smallest library fragments will often be overrepresented in NGS data due to their higher efficiency of amplification during bridge PCR. The double Agencourt AMPure XP bead cleanup included in the QIAseq cfDNA Library Kit protocol helps to ensure a narrow fragment size distribution for accurate qPCR and even sequencing coverage of all library molecules.

Protocol: cfDNA Sample Extraction

This protocol is for the purification of circulating nucleic acids from 1 ml, 2 ml or 3 ml plasma.

Important points before starting

- Green (marked with ■) denotes 1 ml sample volumes of plasma; blue (marked with ◆) denotes 2 ml sample volumes plasma; red (marked with e) denotes 3 ml sample volumes of plasma.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ■ <1 ml, ◆ <2 ml or e <3 ml, bring the volumes up to ■ 1 ml, ◆ 2 ml or e 3 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described on page 17.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.
- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions on page 17.

Procedure

1. Pipet ■ 100 µl, ◆ 200 µl or ● 300 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).
2. Add ■ 1 ml, ◆ 2 ml or ● 3 ml of plasma to the 50 ml tube.
3. Add ■ 0.8 ml, ◆ 1.6 ml or ● 2.4 ml Buffer ACL. Close the cap and mix by pulse-vortexing for 30 s.

   Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add ■ 1.8 ml, ◆ 3.6 ml or ● 5.4 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate-Buffer ACB mixture in the tube for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.
9. Carefully apply the lysate-Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender. Please note that large sample lysate volumes (about 11 ml when starting with 3 ml sample) may need up to 10 minutes to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient

release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini columns.

10. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
11. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.
13. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
14. Place the QIAamp Mini column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
15. Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 40–60 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

    Important: Ensure that the elution buffer AVE is equilibrated to room temperature (15–25°C). If elution is carried out in small volumes (<50 µl), the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream

applications. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

16. Centrifuge in a microcentrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Plasma

This protocol is for the purification of circulating nucleic acids from 4 ml or 5 ml plasma.

Important points before starting

- Blue (marked with a ◆) denotes 4 ml sample volumes of plasma; red (marked with an e) denotes 5 ml sample volumes of plasma.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ◆ <4 ml or e < 5 ml, bring the volumes up to ◆ 4 ml or e 5 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described on page 17.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.
- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions on page 17.

Procedure

1. Pipet ◆ 400 µl or e 500 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).

2. Add 4 ml or 5 ml of plasma to the tube.
3. Add 3.2 ml or 4.0 ml Buffer ACL. Close the cap and mix by pulse-vortexing for 30 s. Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add 7.2 ml or 9 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate-Buffer ACB mixture in the tube for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.
9. Carefully apply the lysate-Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   Please note that large sample lysate volumes (about 20 ml when starting with 5 ml sample) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

   Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini columns.

10. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
11. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.
13. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
14. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
15. Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 40–60 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

    Important: Ensure that the elution buffer AVE is equilibrated to room temperature (15–25°C). If elution is done in small volumes (<50 µl), the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA. Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.
16. Centrifuge in a microcentrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: cfDNA Library Preparation

This protocol is for constructing sequencing libraries from 1–100 ng of cfDNA input for Ion Torrent NGS platforms.

Important points before starting

- QIAseq sequencing adapters are dissolved in duplex buffer and are ready to use. Each well of the 24-plex single-use plate contains equimolar mixes of universal and individual barcode adapters.
- Adapters are fully compatible with all Ion Torrent instruments, including PGM™, Proton and S5 instruments.
- Only the adapters supplied with this kit are compatible with the innovative All-in-One end repair and ligation reaction.
- The majority of circulating cell-free DNA (cfDNA) is around 170 bp in size and does not require further fragmentation prior to library preparation.
- Do not use a heated lid during the adapter ligation step.

End-polishing and adapter ligation

This protocol describes end repair, A-addition, adapter ligation and library cleanup, and generates libraries that are ready to quantify and use in next-generation sequencing on Ion Torrent sequencers.

Things to do before starting

- Quantitate purified double-stranded cfDNA using a fluorometric method. Start with 1– 100 ng cfDNA in Buffer EB, nuclease-free H_2O or 10 mM Tris·HCl (pH 8.0). Thaw frozen reagents on ice. Once thawed, buffers should be mixed thoroughly by quick

vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.

- Sample cfDNA should be dissolved in Buffer AVE, EB/Tris buffer or H_2O before starting.
- Prepare 80% ethanol.

Procedure

1. Before setting up the All-in-One library preparation reaction mix, vortex and spin down the thawed adapter plate.
2. Set up the All-in-One library preparation reaction mix on ice according to Table 2. Remove the protective adapter plate lid. Pierce the foil seal for each well of the adapter plate to be used right before pipetting.

Table 2. All-in-One Library preparation reaction setup

Component	Volume/reaction
Input DNA (cfDNA)	Variable
All-in-One Reaction Buffer, 4x	22.5
All-in-One Enzyme Mix	6
QIAseq Adapter Mix (24-plex plate)	5
Nuclease-free water	Variable
Total reaction volume	**90 µl**

3. Mix by gently pipetting 5–6 times.
4. Load onto the thermal cycler and start the All-in-One reaction program (Table 3).
   Important: Do not use a thermal cycler with a heated lid.

Table 3. End-polishing, adapter ligation and nick repair thermal cycling conditions

Program	Temperature	Time	Additional comments
End-polishing/Ligation	25°C	30 min	Polishing the ends of DNA fragments and adapter ligation
Nick repair	72°C	5 min	Inactivation of end-polishing and ligation enzymes; nick repair
	4°C	∞	Hold

5. When the program is complete, proceed immediately to cleanup.
6. Transfer 90 µl ligation reaction to a 1.5 ml LoBind tube or PCR plate.
7. Add 27 µl resuspended Agencourt AMPure XP beads to each sample and mix.
8. Incubate for 5 min at room temperature.
9. Pellet the beads on a magnetic stand and wait until the solution is clear.
10. Transfer 110 µl supernatant to a new tube or PCR plate. Discard the beads.
11. Add 27µl resuspended Agencourt AMPure XP beads to each sample and mix.
12. Incubate for 5 min at room temperature.
13. Pellet the beads on a magnetic stand and wait until the solution is clear, then carefully discard the supernatant.
14. Add 200 µl fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand and carefully discard the supernatant.
15. Repeat step 14 for a total of 2 ethanol washes. Remove excess ethanol.
16. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying may result in lower DNA recovery. Remove from the magnetic stand.
17. Elute by resuspending in 26 µl Buffer EB. Pellet beads on the magnetic stand. Carefully transfer 23.5 µl supernatant into a new PCR plate.

Amplification of cfDNA library

This protocol is for high-fidelity amplification of the cfDNA library using the QIAseq HiFi PCR Master Mix that is included in the kit. We recommend library amplification prior to sequencing on Ion Torrent instruments to enrich for correct adapter-ligated library molecules.

Things to do before starting

- Prepare library cfDNA using the protocol entitled "End-polishing and adapter ligation"
- Thaw frozen reagents on ice. Once thawed, buffers should be mixed thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.

Procedure

18. Program a thermal cycler with a heated lid according to Table 4.

Table 4. Library amplification cycling conditions

Incubation time	Temperature	Number of cycles
2 min	98°C	1
20 s 30 s 30 s	98°C 60°C 72°C	**Variable, depending on DNA input***: **7** (100 ng input); **10** (10 ng input); **12** (1 ng input)
1 min	72°C	1
∞	4°C	Hold

* Number of PCR cycles depends on input DNA amount and quality.

19. Prepare reactions on ice according to Table 5. Pipet 6–8 times to mix.

Table 5. Amplification reaction setup

Component	Volume/reaction (µl)
Library DNA	23.5
HiFi PCR Master Mix, 2x	25
Primer Mix	1.5
Total reaction volume	**50 µl**

20. Transfer the PCR plate to the thermal cycler and start the program.
21. When the program is complete, remove the plate and add 50 µl of resuspended Agencourt AMPure XP beads to each amplified library.
22. Follow steps 12–16. Elute by resuspending in 27 µl nuclease-free water or Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 25 µl supernatants to new tubes for storage.
23. Assess the library quality using a capillary electrophoresis method. The median library size will be the fragment size plus about 80 bp for the adapters. Libraries can be quantified with qPCR using a QIAseq Library Quant Array (sold separately).
24. Purified libraries can be stored at –20°C until ready for sequencing or hybrid capture.

Protocol: Library QC and Quantification

1. Assess the library quality using a capillary electrophoresis device such as QIAGEN's QIAxcel Advanced or the Agilent Bioanalyzer. Check for expected size distribution and the absence of adapters or adapter-dimers around 80 bp, as well as substantial amounts of unwanted larger library fragments (see Figure 6).

   Note: The median library size will be the fragment size plus 80 bp for the adapters. Libraries can be quantified with qPCR using a QIAseq Library Quant Array (sold separately).

   Note: The median fragment size can be used for subsequent qPCR-based quantification methods to quantify the library concentration (step 2).

2. Quantify the library using the QIAseq Library Quant Array Kit or a comparable qPCR-based method.

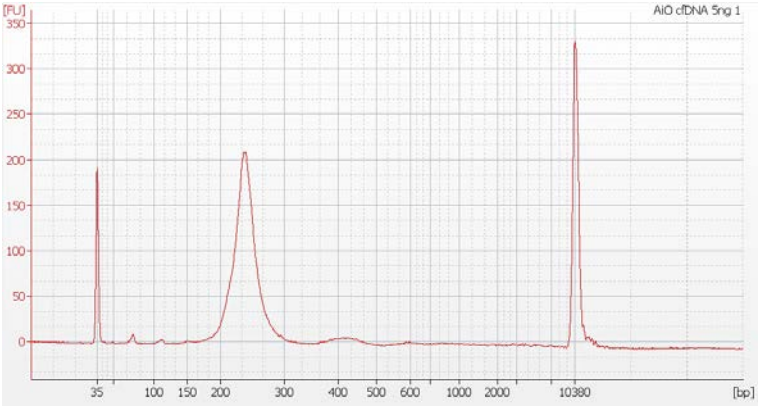


Figure 6. Capillary electrophoresis device trace data showing the correct size distribution of library fragments from cfDNA and the absence of adapters, adapter dimers or larger library fragments.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and / or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

QIAseq cfDNA Extraction Kit

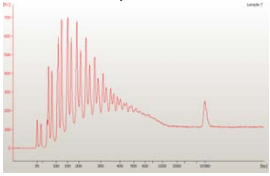
		Comments and suggestions
Little or no nucleic acids in the eluate		
a)	Primary blood tube contains an anticoagulant other than EDTA	Anticoagulants other than EDTA may lead to accelerated DNA degradation compared to EDTA blood. Repeat the purification procedure with new samples.
b)	Extended time between blood draw and plasma preparation	Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid.
c)	Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided as this may lead to DNA degradation. Always use fresh samples or samples thawed only once.
d)	Low concentration of target DNA in the samples	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples.
e)	Inefficient sample lysis in Buffer ACL	If QIAGEN Proteinase K was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh QIAGEN Proteinase K.
f)	Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which may contain methanol or methyl ethyl ketone.
g)	Buffer ACB prepared incorrectly	Check that Buffer ACB concentrate was reconstituted with the correct volume of isopropanol (not ethanol).

		Comments and suggestions
h)	Buffer ACW1 or Buffer ACW2 prepared incorrectly	Check that Buffer ACW1 and Buffer ACW2 concentrates were diluted with the correct volume of ethanol (see page 17). Repeat the purification procedure with new samples.
i)	Buffer ACW1 or Buffer ACW2 prepared with 70% ethanol	Check that Buffer ACW1 and Buffer ACW2 concentrates were diluted with 96–100% ethanol (see page 17). Repeat the purification procedure with new samples.
DNA does not perform well in downstream library preparation		
a)	Little or no DNA in the eluate	See "Little or no nucleic acids in the eluate" above for possible reasons. Increase the amount of eluate added to the reaction if possible.
b)	Inappropriate elution volume used	Using elution volumes of 40–60 µl is optimal for downstream library preparation protocol. Small elution volumes lead to high percentage of sample loss on extraction column, too high elution volumes may lead to highly diluted DNA and thereby limit the percentage of DNA sample that can be used for library generation.
c)	Buffers not mixed thoroughly	Salt and ethanol components of wash Buffer ACW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
General handling		
a)	Clogged QIAamp Mini column	Close the VacValve, if used, and carefully remove the whole assembly consisting of tube extender, QIAamp Mini column, VacConnector and VacValve from the QIAvac 24 Plus manifold. Carefully transfer the remaining sample lysate from the tube extender to a new 50 ml tube. Remove the QIAamp Mini column from the assembly (see above), place it in a 2 ml collection tube and spin it at full speed for 1 minute or until sample has completely passed through the membrane. Re-assemble the QIAamp Mini column with Tube Extender, VacConnector and (optional) VacValve. Transfer the remaining sample lysate into the Tube Extender, switch on the vacuum pump, open the VacValve, and pass the remaining lysate through the QIAamp Mini column. Repeat the above procedure if the QIAamp Mini column continues to clog. Cryoprecipitates may have formed in plasma due to repeated freezing and thawing. These can block the QIAamp Mini column. Do not use plasma that has been frozen and thawed more than once.
b)	Variable elution volumes	Different samples can affect the volume of the final eluate. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

		Comments and suggestions
c)	Vacuum pressure of 800–900 mbar not reached	The vacuum manifold is not tightly closed. Press down on the lid of the vacuum manifold after the vacuum is switched on. Check if vacuum pressure is reached. Gasket of QIAvac lid has worn out. Check the seal of the manifold visually and replace it if necessary. VacValves have worn out. Remove all VacValves and insert VacConnectors directly into the luer extensions. Insert QIAamp Mini columns into VacConnectors, close the lid of the columns and switch on vacuum. Check if vacuum pressure is reached. Replace VacValves if necessary.

QIAseq cfDNA Library T Kit

		Comments and suggestions
Low library yields		
a)	Suboptimal reaction conditions due to low DNA quality	Make sure to use the highest-quality sample DNA available to ensure optimal activity of the library enzymes.
b)	Overdrying of the Agencourt AMPure XP beads during cleanup steps.	Overdrying of the Agencourt AMPure XP beads can make it difficult to elute the DNA off the beads. Do not dry beads for more than 10 min at room temperature.
Unexpected signal peaks in capillary electrophoresis device traces		
a)	Presence of shorter peaks between 60 and 120 bp	These peaks represent library adapters and adapter dimers that occur when there is no, or insufficient, adapter depletion after library preparation. Adapter dimers can be similar to regular library fragments and also bind to Ion Sphere™ Particles but will not be efficiently amplified during emulsion PCR. This results in higher numbers of blank beads and will reduce the sequencing capacity of the chip. A low ratio of adapter dimers versus library will not be a problem. Make sure the correct volume of the Agencourt AMPure XP beads is used for the cleanup steps.
b)	Presence of larger library fragments after library enrichment	If the fragment population shifts higher than expected, this can also be due to the carry-over of the Agencourt AMPure XP beads. Make sure not to aspirate beads while taking supernatant during the cleanup steps.
c)	Incorrect library fragment size after adapter ligation	During library preparation, adapters of approximately 40 bp are ligated to both ends of the DNA library fragments. This should be reflected on a capillary electrophoresis device by a shift in size of all library fragments of 80 bp.

		Comments and suggestions
d)	Multiple unspecific shorter fragments after ligation	Make sure to use the single-use adapter mixes supplied with the QIAseq cfDNA All-in-One Kit have been used in the reaction. Patterns of narrow peaks of shorter fragments (see below) indicate that adapters have been used that are incompatible with the All-in-One library prep reaction. 

Appendix A: Recommendation for Plasma Separation and Storage

In order to isolate circulating, cell-free nucleic acids from blood samples, we recommend following this protocol, which includes a high *g*-force centrifugation step to remove cellular debris and thereby reduce the amount of cellular or genomic DNA in the sample.

A1. Place whole EDTA blood in BD Vacutainer™ tubes (or other primary blood tubes containing EDTA as anti-coagulant) in a centrifuge with swinging-out rotor and appropriate buckets.

A2. Centrifuge blood samples for 10 min at 1900 x *g* (3000 rpm) and 4°C temperature setting.

A3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer. About 4–5 ml plasma can be obtained from a single 10 ml primary blood tube.

Note: Plasma can be used for circulating nucleic acid extraction at this stage.

However, the following high-speed centrifugation will remove additional cellular debris and contamination of the circulating nucleic acids by gDNA and RNA derived from damaged blood cells.

A4. Transfer aspirated plasma into fresh 15 ml centrifuge tubes with a conical bottom.

A5. Centrifuge plasma samples for 10 min at 16,000 x *g* (in a fixed-angle rotor) and 4°C temperature setting. This will remove additional cellular nucleic acids attached to cell debris.

A6. Without disturbing the pellet, carefully remove supernatant to a new tube with a pipet.

A7.If plasma will be used for nucleic acid extraction on the same day, store at 2–8°C until further processing. For longer storage, keep plasma frozen at –80°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature.

In case of cryoprecipitates, follow these two steps:

- To remove cryoprecipitates, centrifuge plasma sample for 5 min at 16,000 x *g* (in a fixed angle rotor) and 4°C temperature setting.
- Remove supernatant to a new tube, and then begin with the nucleic acid extraction protocol.

Appendix B: Adapter Barcodes for 24-plex Adapter Plates

The barcode sequences used in the QIAseq cfDNA Library T Kit 24-plex adapter plate are listed in Table 6. The layout of the 24-plex single use adapter plate is displayed in Figure 7.

Table 6. Adapter barcodes used in the QIAseq cfDNA Library T Kit adapter plate

Barcode adapter name	Barcode sequence
Ion Torrent Barcode 1	CTAAGGTAAC
Ion Torrent Barcode 2	TAAGGAGAAC
Ion Torrent Barcode 3	AAGAGGATTC
Ion Torrent Barcode 4	TACCAAGATC
Ion Torrent Barcode 5	CAGAAGGAAC
Ion Torrent Barcode 6	CTGCAAGTTC
Ion Torrent Barcode 7	TTCGTGATTC
Ion Torrent Barcode 8	TTCCGATAAC
Ion Torrent Barcode 9	TGAGCGGAAC
Ion Torrent Barcode 10	CTGACCGAAC
Ion Torrent Barcode 11	TCCTCGAATC
Ion Torrent Barcode 12	TAGGTGGTTC
Ion Torrent Barcode 13	TCTAACGGAC
Ion Torrent Barcode 14	TTGGAGTGTC
Ion Torrent Barcode 15	TCTAGAGGTC
Ion Torrent Barcode 16	TCTGGATGAC
Ion Torrent Barcode 17	TCTATTCGTC
Ion Torrent Barcode 18	AGGCAATTGC
Ion Torrent Barcode 19	TTAGTCGGAC
Ion Torrent Barcode 20	CAGATCCATC
Ion Torrent Barcode 21	TCGCAATTAC
Ion Torrent Barcode 22	TTCGAGACGC
Ion Torrent Barcode 23	TGCCACGAAC
Ion Torrent Barcode 24	AACCTCATTC

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC1/Universal	BC9/Universal	BC17/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
B	BC2/Universal	BC10/Universal	BC18/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
C	BC3/Universal	BC11/Universal	BC19/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
D	BC4/Universal	BC12/Universal	BC20/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
E	BC5/Universal	BC13/Universal	BC21/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
F	BC6/Universal	BC14/Universal	BC22/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
G	BC7/Universal	BC15/Universal	BC23/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty
H	BC8/Universal	BC16/Universal	BC24/Universal	empty	empty	empty	empty	empty	empty	empty	empty	empty

Figure 7. Single-use 24-plex adapter plate layout with equimolar mixes of universal adapter and individual barcode adapters. Columns 4–12 remain empty.

Appendix C: Library Quantification and Quality Control

Quality control for the library construction process can be performed using QIAGEN's QIAseq Library Quant Array Kit (cat. no. 333304). With this assay, the correct dilution of the library can also be determined for sequencing. Please refer to the corresponding handbook for library quantification and quality control.

Ordering Information

Product	Contents	Cat. no.
QIAseq cfDNA All-in-One T Kit (24)	For 24 reactions on Ion Torrent sequencers: QIAamp Mini Columns, tubes, reagents and buffers for cfDNA extraction for NGS, enzymes and buffers for cfDNA library prep, Ion Torrent Adapter Plate 24-plex, Ion Torrent Library Amplification Primer and PCR Master Mix	180043
Related product		
QIAseq Library Quant Array Kit	Laboratory-verified forward and reverse primers for 500 x 25 µl reactions (500 µl); DNA Standard (100 µl); Dilution Buffer (30 ml); (1.35 ml x 5) GeneRead™ qPCR SYBR® Green Mastermix	333304

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Limited License Agreement for the QIAseq cfDNA All-in-One T Kit (24).

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