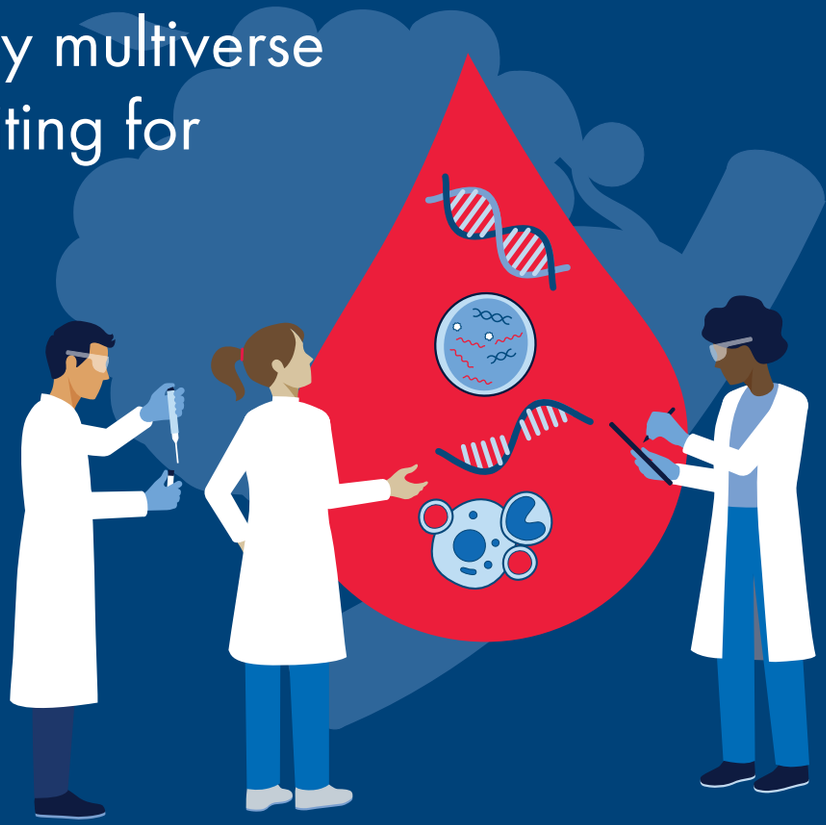
A multiverse of liquid biopsy insights

Multianalyte liquid biopsy technical guidelines

For parallel isolation of CTC nucleic acids, cell-free DNA and EV-derived circulating RNA from blood samples

Sample to Insight

The liquid biopsy multiverse is out there, waiting for you to explore.



Imagine an innovative method of understanding the process of cancer to provide crucial insights for a personalized disease management without invasive and painful tissue biopsy procedures – that's liquid biopsy. This revolutionary approach to cancer research uses body fluids to analyze a wide range of omics-based biomarkers to give a comprehensive view of cancer.

Compared with tissue biopsy, liquid biopsy is minimally invasive, repeatable, can enable early cancer detection and is more relevant to individuals with a tumor mass that is difficult to reach and/or has metastasized. Additionally, liquid biopsy better mirrors the heterogeneity of the cancer disease and provides complementary real-time information on the cancer evolution, therapeutic response and minimal residual disease.

Typically, only one liquid biopsy analyte (LBA) is investigated in a blood sample. By doing so, the full potential of liquid biopsy is not maximized, since after the extraction of the target nucleic acid, precious leftover samples are discarded. In this manner, researchers miss out on potentially crucial genomic insights that can be obtained from the same blood draw.

In recent years, there has been a growing interest in multianalyte liquid biopsy research, where multiple LBAs are extracted and investigated from the same blood draw. This is done to optimize usage of precious samples, to generate a more complete picture of cancer, and to compare insights in a more meaningful manner.

In order to support the standardization of parallel isolation of multiple LBAs in liquid biopsy samples, we developed a comprehensive multianalyte isolation workflow. Herein, we provide a technical guide with step-by-step information on how to easily implement the workflow in your laboratory.

What are the advantages of **multianalyte** liquid biopsy research?

- **It makes the most out of your precious blood samples.**
  By isolating multiple LBAs, precious blood samples are not discarded, thus potentially maximizing the amount of insights generated.

- **It gives a more complete picture of cancer.**
  A single LBA is not enough to show the entire picture of cancer. Each LBA isolated from the same blood draw complement each other to shed light on the cancer disease process at different omics levels.

- **It can be tailored to your own pace in order to allow convenient planning of laboratory work.**
  There is no need to isolate all of the LBAs at the same time. You can store your samples at specific steps during the workflow, allowing you to complete your research project at your own pace and plan your study ahead of time.

Which LBAs can be isolated with the multianalyte liquid biopsy workflow?

Various body fluids and target LBAs can be investigated in liquid biopsy research. For this guide, the analytes mentioned are based on the Evaluation of multiple Liquid biopsy analytes In Metastatic breast cancer patients All from one blood sample (ELIMA) study (1). QIAGEN offers Sample to Insight solutions designed for target enrichment, isolation and analysis.

Circulating tumor–cell–derived nucleic acids acids

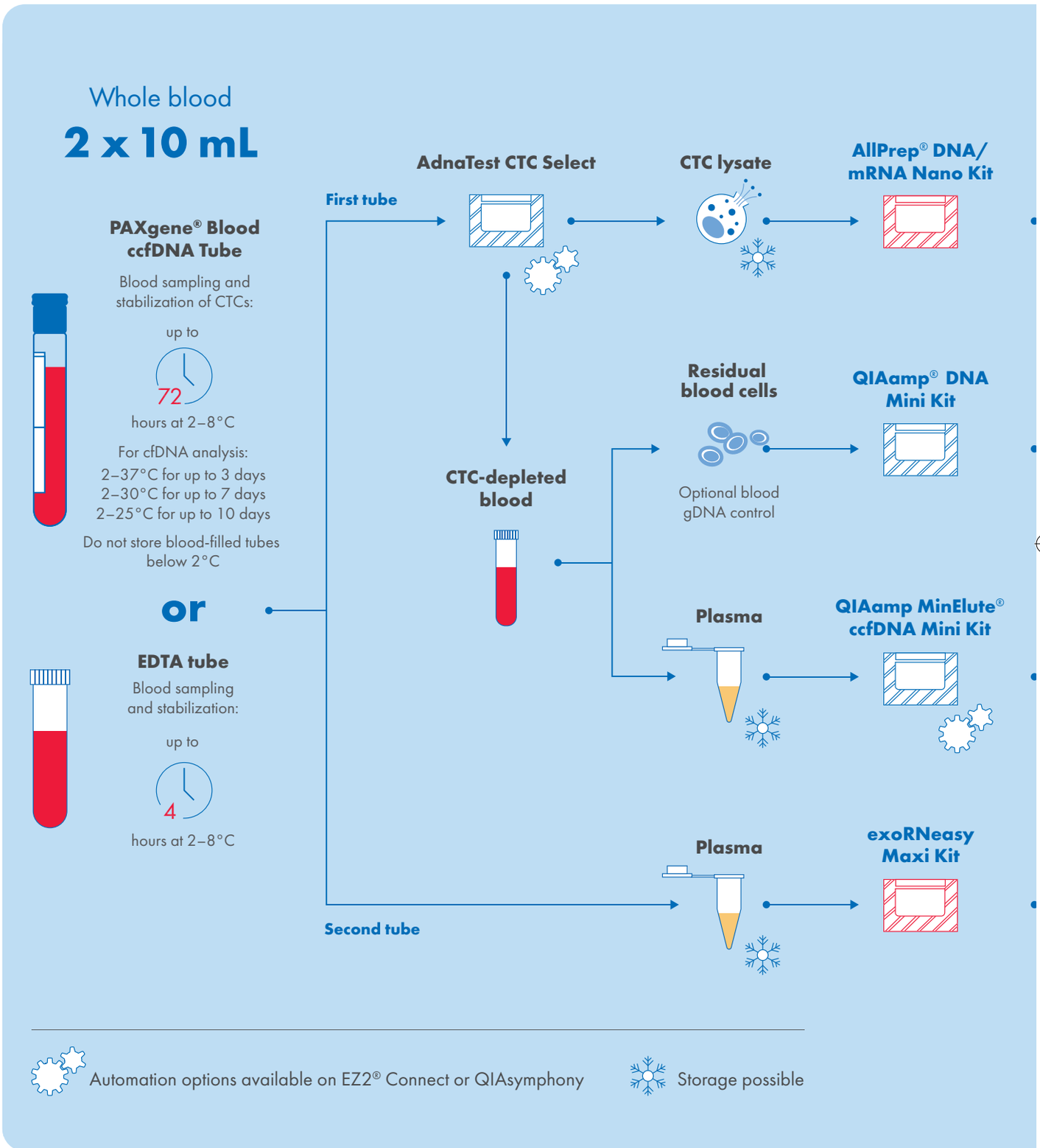
Cell–free DNA (cfDNA)

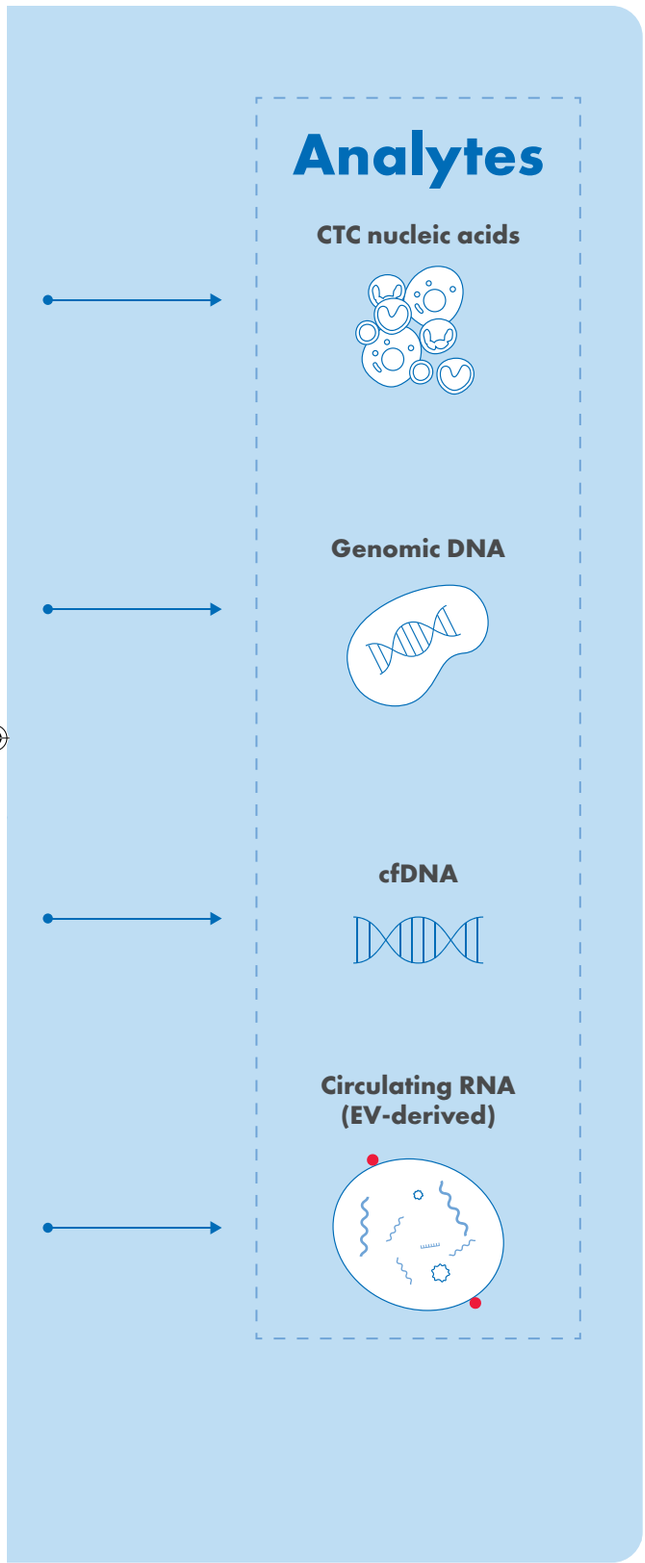
Extracellular–vesicle (EV)–derived Circulating RNAs

However, multianalyte liquid biopsy research is not limited to the isolation and analysis of these three LBAs. Additional analysis of genomic DNA (gDNA) from peripheral blood mononuclear cells (PBMCs) and circulating miRNA can be done with QIAGEN Sample to Insight solutions. To learn more, visit **qiagen.com/MultianalyteLB**

Note: The multianalyte liquid biopsy technical guide is intended for molecular biology applications. The products mentioned in this guide is not intended for the diagnosis, prevention or treatment of a disease.

Multianalyte liquid biopsy workflow overview





Safety Information

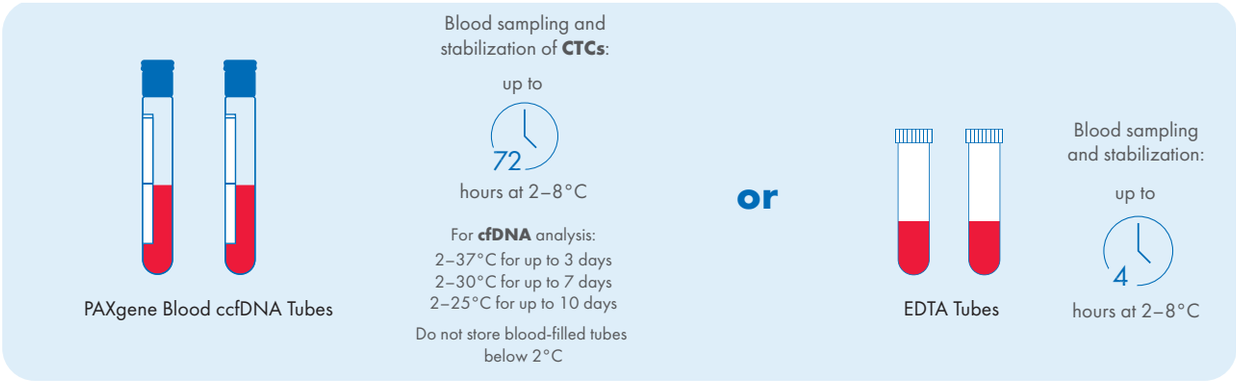
When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view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

Important Note

The multianalyte liquid biopsy technical guide is not a replacement for the respective QIAGEN kit handbooks. This user guide is intended to provide a birds-eye view of the multianalyte workflow. For the detailed steps in every protocol, please follow the steps in the respective QIAGEN handbook for the specific analyte you are trying to isolate.

Multianalyte Liquid Biopsy Workflow

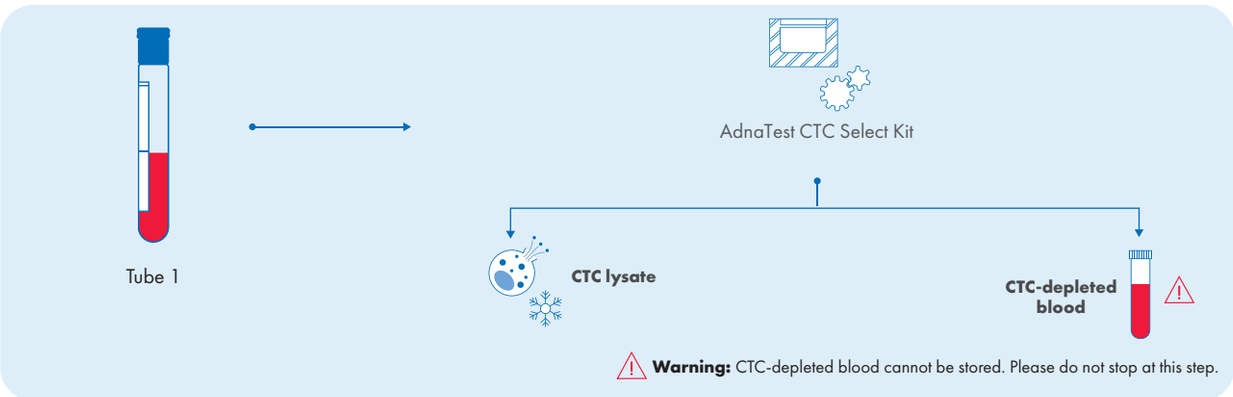
Blood collection and stabilization



Before starting this workflow, you will need as little as 20 mL whole blood. It can be sampled in 2 EDTA tubes or in 2 PAXgene Blood ccfDNA Tubes. We recommend using the PAXgene Blood ccfDNA Tube to minimize the release of DNA into plasma and stabilize your samples during transport and storage. Additionally, you can benefit from a formaldehyde-free, non–crosslinking stabilization of the circulating cfDNA in plasma and genomic DNA in the nuclear cellular fraction.

Part 1: Enrichment of CTCs from whole blood and isolation of CTC nucleic acids

Part 1A: Enrichment of CTCs using AdnaTest CTC Select Kit



1. Follow steps 1 to 6 of *Procedure A: Preparation of the Select Beads* from the **AdnaTest CTC Select Handbook**.

Download the AdnaTest CTC Select Kit Handbook here: **www.qiagen.com/HB-2519**

2. Take 5 mL of blood sample from Tube 1 to isolate CTCs with the AdnaTest CTC Select Kit.

Note: CTC isolation requires fresh or stabilized blood samples.

Note: You can use the remaining 5 mL of blood sample to duplicate the CTC enrichment steps or to extract plasma.

3. Resume with steps 2 to 6 of *Procedure B: Selection of tumor cells* from the **AdnaTest CTC Select Handbook.**

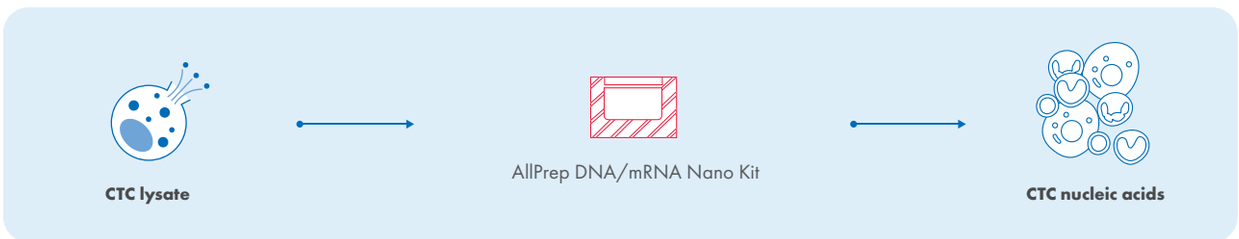
4. Use the CTC–depleted blood from 5 mL blood sample for "Part 2: Isolation of cfDNA using QIAamp MinElute ccfDNA Kit".
   Note: Do not store the CTC–depleted blood. Use it as soon as possible.

5. Continue with steps 7 to 20 of *Procedure B: Selection of tumor cells* from the **AdnaTest CTC Select Handbook.**

Note: The lysate containing CTCs can be stored for up to 4 weeks at −30 to −15°C, allowing you a to conveniently plan your research project.

Note: Alternatively, you can automate the CTC enrichment on the EZ2 Connect using the EZ2 AdnaTest CTC Select Kit. To learn more, please visit **www.qiagen.com/EZ2AdnaTest**

Part 1B: Isolation and purification of CTC Nucleic Acids Using AllPrep DNA/mRNA Nano Kit

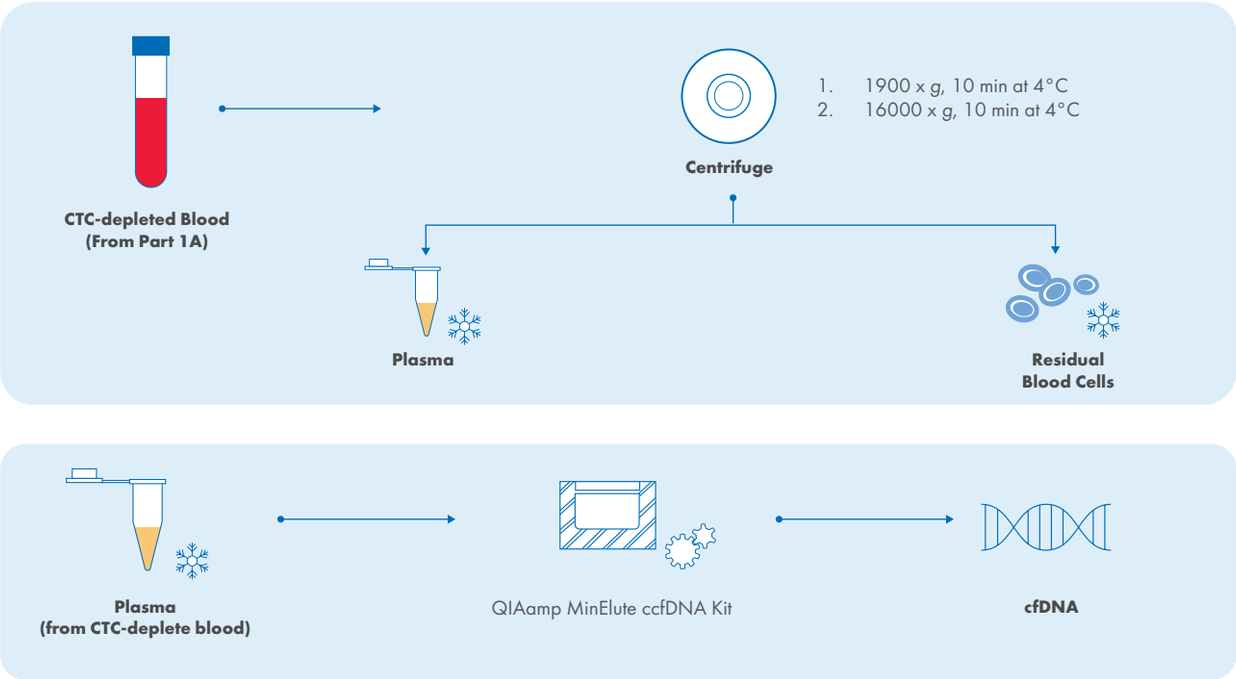


1. Follow the steps in *Protocol: Simultaneous purification of genomic DNA and mRNA from low-biomass samples* from the **AllPrep DNA/mRNA Nano Handbook.**

Download the AllPrep DNA/mRNA Nano Kit Handbook here: **www.qiagen.com/HB-2772**

Note: You can store the extracted genomic DNA and mRNA at −30 to −15°C for up to 4 weeks for later analysis.

Part 2: Isolation of cfDNA using QIAamp MinElute ccfDNA Kit

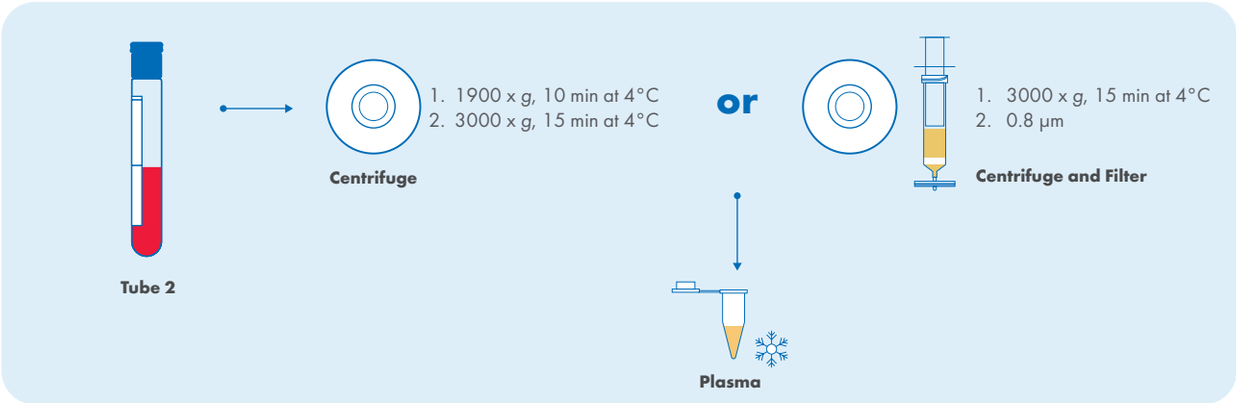


1. Use the pooled CTC-depleted blood from part 1A, step 5.
2. When using EDTA tubes, centrifuge at 1900 *x g* for 15 minutes at 4°C with an optional second centrifugation step at 1900 *x g* for 15 minutes at 4°C.
   Note: If using the PAXgene Blood ccfDNA Tube, please refer to the protocol mentioned in the PAXgene Blood ccfDNA Tube Handbook. For more information.
   Visit **www.qiagen.com/PAXgeneBloodccfDNA**
3. Transfer 4 mL of plasma into a separate tube.
   Optional: Reserve the residual blood cells (PBMCs) to isolate gDNA using the QIAamp DNA Mini Kit. To learn more please visit **www.qiagen.com/QIAampDNAKits**
   Note: The plasma containing cfDNA can be stored for up to 4 weeks at −30 to −15°C and more than a month at −90 to −65°C allowing to conveniently plan of your research project.
4. Follow steps 1 to 11 of *Protocol: Purification of circulating DNA from 1–4 mL serum or plasma using the QIAamp MinElute ccfDNA Mini Kit* from the **QIAamp MinElute ccfDNA Handbook**.

Download the QIAamp MinElute ccfDNA Kit here: **www.qiagen.com/HB-2366**

Note: Alternatively, you can automate ccfDNA isolation on the EZ2 Connect using the EZ1&2® ccfDNA Kit or on the QIAsymphony using the QIAsymphony PAXgene Blood ccfDNA Kit. To learn more, please visit **www.qiagen.com/EZ2ccfDNA** or **www.qiagen.com/QIAsymphonyccfDNA**

Part 3: Isolation of EV-derived circulating RNA using the exoRNeasy Maxi Kit



1. Use the second tube containing 10 mL of whole blood sample.
2. Centrifuge at 1900 x *g* for 10 minutes to capture the larger EVs at 4°C (but excluding the apoptotic bodies). To capture the smaller vesicles, centrifuge for a second time at 3000 x *g* for 15 minutes at 4°C or use a syringe filter with a pore size of 0.8 µm.
   Note: If using the PAXgene Blood ccfDNA Tube in extracting EV-derived Circulating RNA, please refer to the protocol mentioned in the PAXgene Blood ccfDNA Tube Handbook (the same steps in the section Plasma Preparation Isolation of ccfDNA is also applicable for EV-derived circulating RNA). For more information visit **www.qiagen.com/PAXgeneBloodccfDNA**
3. Transfer 4 mL of plasma into a separate collection tube.
   Note: The plasma containing EV-derived circulating RNA can be stored for up to 4 weeks at −30 to −15°C and more than a month at 90 to −65°C allowing you to conveniently plan your research project.
4. Follow steps 1 to 18 of Protocol: Purification of total *exosomal RNA, including miRNA, from serum and plasma* from the **exoRNeasy Midi/Maxi Handbook**.

Download the exoRneasy Maxi Kit Handbook here: **www.qiagen.com/HB-2630**

Optional: Alternatively, the second blood tube can be used to isolate circulating miRNA from plasma or serum using the miRNeasy Serum/Plasma Advanced Kit.
To learn more, please visit **www.qiagen.com/miRNeasySPAdvanced**

References

1. Keup C., Kimmig R & Kasimir-Bauer S (2022) Multimodality in liquid biopsy: does a combination uncover insights undetectable in individual blood analytes? Journal of Laboratory Medicine, 46(4), 255-264.
2. Keup C, Suryaprakash V, Storbeck M, Hoffmann O, Kimmig R, Kasimir-Bauer S. Longitudinal Multi-Parametric Liquid Biopsy Approach Identifies Unique Features of Circulating Tumor Cell, Extracellular Vesicle, and Cell-Free DNA Characterization for Disease Monitoring in Metastatic Breast Cancer Patients. Cells. 2021;10(2):212.
3. Keup C, Suryaprakash V, Hauch S, et al. Integrative statistical analyses of multiple liquid biopsy analytes in metastatic breast cancer. Genome Med. 2021;13(1):85.
4. Keup C, Storbeck M, Hauch S, et al. Multimodal Targeted Deep Sequencing of Circulating Tumor Cells and Matched Cell-Free DNA Provides a More Comprehensive Tool to Identify Therapeutic Targets in Metastatic Breast Cancer Patients. Cancers (Basel). 2020;12(5):1084.
5. Keup C, Benyaa K, Hauch S, et al. Targeted deep sequencing revealed variants in cell-free DNA of hormone receptor-positive metastatic breast cancer patients. Cell Mol Life Sci. 2020;77(3):497-509.
6. Maltoni R, Palleschi M, Ravaioli S, et al. Cell-Free DNA Variant Sequencing Using CTC-Depleted Blood for Comprehensive Liquid Biopsy Testing in Metastatic Breast Cancer. Cell Transplant. 2020;29:963689720925057.
7. Keup C, Mach P, Aktas B, et al. RNA Profiles of Circulating Tumor Cells and Extracellular Vesicles for Therapy Stratification of Metastatic Breast Cancer Patients. Clin Chem. 2018;64(7):1054-1062.

Ordering Information

Product	Contents	Cat. no.
PAXgene Blood ccfDNA Tube*	For collection of whole blood samples and stabilization of circulating cell-free DNA (ccfDNA) from plasma and genomic DNA (gDNA) from the nucleated cellular fraction	768115
AdnaTest CTC Select Kit	For the enrichment and molecular characterization of circulating tumor cells (CTCs) from whole human blood	395092
AllPrep DNA/mRNA Nano Kit	For the simultaneous purification of genomic DNA (gDNA) and messenger RNA (mRNA) from low-biomass samples	80272
QIAamp MinElute ccfDNA Mini Kit	For isolation of free-circulating DNA from human plasma or serum	52284
exoRNeasy Maxi Kit	For efficient isolation of exosomal RNA from biofluids	77164
QIAamp DNA Mini Kit (50)	For isolation of genomic DNA	51304
miRNeasy Serum/Plasma Advanced Kit	For purification of cell-free total RNA, including miRNA, from animal and human plasma and serum	217204
EZ2 Connect	For end-to-end automation of nucleic acid extraction, from reagent setup to elution	9003210
QIAsymphony SP	For fully automated DNA/RNA purification from a broad range of samples with varying input volumes	9001297
EZ2 AdnaTest CTC Select Kit	For automated immunomagnetic enrichment and integrated mRNA isolation for molecular characterization of circulating tumor cells (CTCs) from whole human blood	395692
EZ1&2 ccfDNA Kit	For automated isolation of cell-free (cfDNA) DNA from human plasma or serum on EZ1 Advanced XL or EZ2 Connect	954854
QIAsymphony PAXgene Blood ccfDNA Kit	For purification of ccfDNA from plasma using the QIAsymphony SP	768536

* Available in United States; not available in all countries. For Research Use Only. Not for use in diagnostic procedures.

To learn more about Multianalyte Liquid Biopsy, visit **www.qiagen.com/multianalyteLB**

The Multianalyte Liquid Biopsy Technical Guide is intended for molecular biology applications. The products mentioned in this guide is not intended for the diagnosis, prevention, or treatment of a disease.

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

Ordering	**www.qiagen.com/shop**
Technical Support	**www.support.qiagen.com**
Website	**www.qiagen.com**