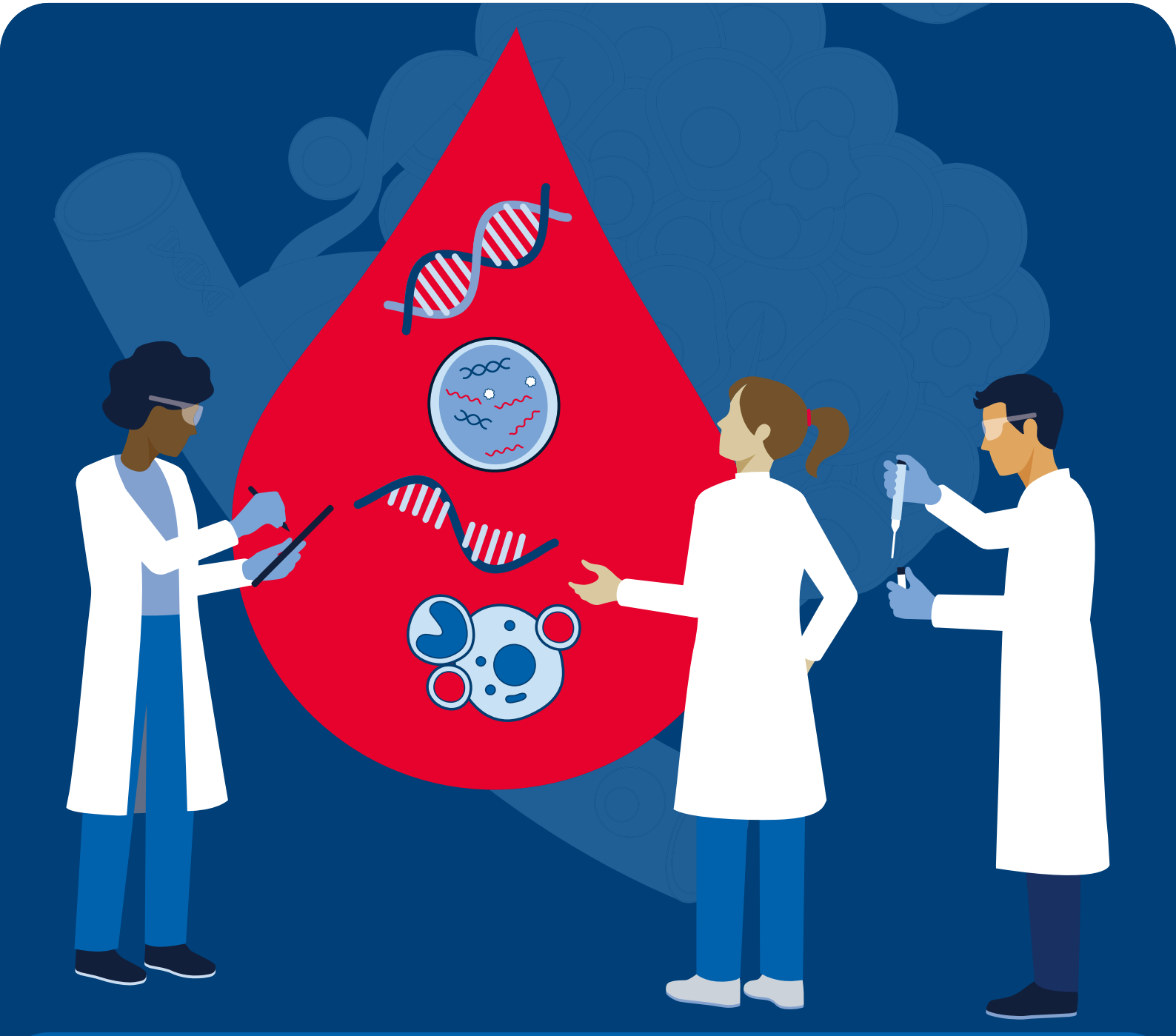
Liquid Biopsy

Accelerate Your Circulating Biomarker Research

QIAGEN



Sample to Insight

Cross the bridge from discovery to insight

Liquid biopsy is a non-invasive approach to help you detect circulating tumor cells and disease biomolecules in blood, urine and other body fluids. It is a useful method when:

- Your tissue sample is limited
- Your sample contains insufficient tumor tissue
- Tumors are hard to reach
- Regular monitoring is needed

Liquid biopsy begins with sample collection and processing and demands high efficiency for successful down-stream detection. To unlock the molecular biomarkers in body fluids, we provide sample processing solutions for all major liquid biopsy approaches – including automation.

A workflow with optimized next-generation sequencing (NGS) library solutions ensures the most accurate and reproducible analysis for DNA, RNA, and miRNA as well as multi-modal analysis of DNA and RNA together.

Nanoplate-based digital PCR technology and dedicated assays allow the detection and quantification of rare mutations in liquid biopsies in an extremely sensitive, precise and reproducible way.

QIAGEN® Digital Insights solutions combine state-of-the-art data analysis and interpretation. Optimized, stream-lined end-to-end workflows enable high sensitivity in detection, filtering and interpretation of variants.

Sample collection

- Ensure long-term stability of markers
- Eliminate analysis errors caused by transportation and storage

cfDNA

- PAXgene® Blood ccfDNA Tubes

Sample preparation

- Explore the biomarker universe with circulating nucleic acids, extracellular vesicles and CTCs
- Achieve highest yields and purity from precious biofluid samples

cfDNA

- QIAamp® MinElute® ccfDNA Kits
- EZ1&2™ ccfDNA Kit
- QIAsymphony® PAXgene Blood ccfDNA Kit*†
- QIAamp Circulating Nucleic Acid Kit

cfRNA & Exosomes

- miRNeasy Serum/Plasma Advanced Kit
- exoRNeasy Serum/Plasma Kit
- exoEasy Maxi Kit

Circulating tumor cells

- AdnaTest CTC-Select
- EZ2® AdnaTest CTC-Select
- AllPrep® DNA/mRNA Nano Kit

Multianalytes

- See page 13 .

NGS analysis

- Discover your biomarkers with best-of-class platform-agnostic NGSsolutions
- Benefit from unbiased library preparation or targeted panels that ensure the right fit for your applications
- Confidently detect ultrarare cfDNA variants

DNA sequencing

- QIAseq® Targeted cfDNA Ultra Panels:
  - Actionable Solid Tumor Panel
  - Breast Cancer Panel
  - Colorectal Cancer Panel
  - Lung Cancer Panel
  - Myeloid Neoplasms Panel
- QIAseq Ultralow Input Library Kit

miRNA sequencing

- QIAseq miRNA Library Kits

RNA sequencing

- QIAseq FastSelect RNA Library Kits

Total nucleic acid sequencing

- QIAseq Multimodal Panels

Circulating tumor cell mutation profiling

- QIAseq Single Cell DNA library Kits
- QIAseq Human Exome Kits

Circulating tumor cell gene expression profiling

- QIAseq Single Cell RNA Library Kits

Quality control

- Quickly and easily assess sample concentration, purity and size distribution from minimal input volumes
- Assess integrity with user-independent RNA integrity score (RIS)
- Save time and costs and gain peace of mind by processing only the samples of the highest quality

DNA and RNA

- QIAxcel® Connect System

qPCR analysis / dPCR analysis

- Identify disease-relevant gene expression signatures
- Achieve meaningful results with wet bench-validated assays

miRNA biomarker profiling

- miRCURY® LNA® PCR system

Gene expression profiling (mRNA, lncRNA)

- QuantiNova® LNA PCR Assays and Panels
- QuantiNova PCR Kits

Circulating tumor cells

- AdnaTest Add-Ons

- Identification and absolute quantification of mutations
- Biomarker validation
- MRD/resistance monitoring
- Orthogonal validation of NGS data
- Time-efficient, very sensitive and reproducible

QIAcuity dPCR system

- QIAcuity nanoplates and accessories
- QIAcuity instruments

Mutation and CNV analysis

- dPCR LNA mutation assays
- dPCR CNV Probe Assays
- Pan-cancer dPCR panels: Coming soon

miRNA biomarker profiling

- miRCURY LNA® miRNA PCR Assays for Digital PCR

Gene expression

- QuantiNova® LNA PCR Assays for Digital PCR

Data analysis

- Identify variants and determine changes in expression with high accuracy
- Validate results with integrated visualization

- CLC Genomics Workbench
- GeneGlobe Design & Analysis Hub

Interpretation

- Generate biological hypotheses
- Identify and prioritize variants for follow-up

- QCI Interpret
- Ingenuity Pathway Analysis (IPA®)

Genomic Services

- Extend your in-house resources quickly and conveniently
- Get high-quality data using our expertise and robust technologies
- Get tailored and flexible support for the entire experimental journey
- Partner with us for trusted guidance and benefit from the expert consultation

* Not available in all countries.
† Customized protocols for large volumes available (only for molecular biology applications).

Standardize your preanalytical workflow by ensuring accurate preservation

Collection, stabilization, transport and storage

PAXgene Blood ccfDNA Tubes

Reflect the exact level and integrity of circulating, cell-free DNA (cfDNA) at the time of collection by improving the quality, accuracy and reliability of the blood sample.

PAXgene Blood ccfDNA Tubes contain an additive that prevents gDNA release to effectively stabilize cfDNA levels in plasma by minimizing downstream impact and increasing analytical sensitivity (Figure 1). Variability due to different shipping or storage conditions is reduced.

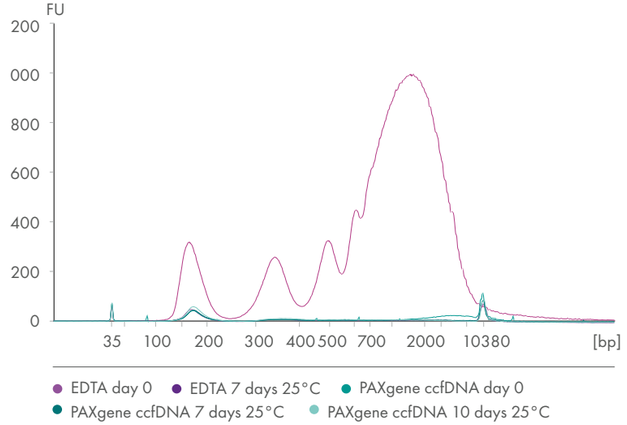


Figure 1. PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into plasma.
Whole blood was stored in EDTA tubes or PAXgene Blood ccfDNA Tubes (CE-IVD)*. cfDNA was purified from the plasma immediately following blood collection (Day 0) and after 7 or 10 days storage at 25°C. Eluate (1 µL) was analyzed using the Agilent High Sensitivity DNA Kit. After 7 days storage, plasma from EDTA tubes showed an increase in apoptotic gDNA fragments, whereas plasma from PAXgene Blood ccfDNA Tubes (CE-IVD) showed a cfDNA profile comparable to day 0.

You can pair PAXgene Blood ccfDNA Tubes seamlessly with the QIAamp Circulating Nucleic Acid Kit, QIAamp MinElute ccfDNA Kits or EZ1&2 ccfDNA Kit. Or you can benefit from a fully validated, integrated and automated solution, optimized protocols and the possibility for direct processing of plasma within primary tubes after one centrifugation step using the QIAsymphony PAXgene Blood ccfDNA Kit.

* CE marked for In Vitro Diagnostic Use according to the Regulation (EU) 2017/746 on In Vitro Diagnostic Medical Devices (IVDR)

Unique formaldehyde-free, non-crosslinking stabilization reagent for reliable, high-sensitivity qualitative analysis

- Blood filled tubes can be stored at room temperature (15–25°C). See performance characteristics for cfDNA stability and gDNA yield, purity and integrity of blood samples stored refrigerated (2–8°C), at room temperature (15–25°C), at 30°C, or at 37°C (Figure 2).
- No DNA modification: Compatible with different downstream assays, including methylation-based assays
- Maximize assay sensitivity by avoiding missing critical mutations*

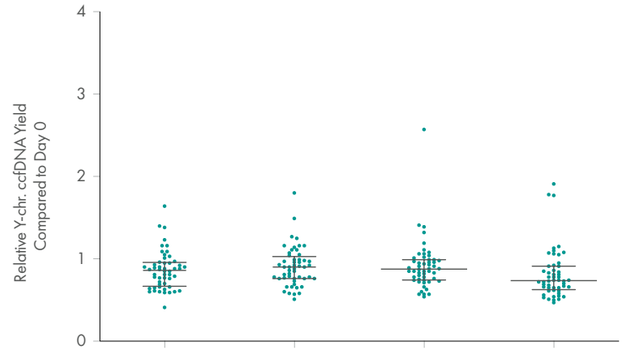


Figure 2. Preservation of cfDNA yield in plasma from blood samples stored in PAXgene Blood ccfDNA Tubes (CE-IVD).
Change in Y-chromosomal male plasma cfDNA yield after whole blood sample storage in comparison to plasma separated within 2 hours of blood collection (Day 0). Blood was drawn from 10 male and 26 female consented, apparently healthy donors. Male blood was processed within 2 hours of blood collection. Whole blood samples were stored at various temperatures for the indicated number of days followed by tube centrifugation and cfDNA purification from plasma using the QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) on the QIAsymphony SP instrument. The relative cfDNA yield was calculated as the ratio of the DYS14 copy numbers after sample storage compared to the copy numbers at Day 0. Medians and the 25th and 75th percentiles are denoted.

Stabilize red blood cells and minimize hemolysis during whole blood storage:

- Maximize plasma recovery and mitigate the risk of gDNA background
- Robust against inappropriate handling and elevated levels of physiological substances
- Eliminate a second centrifugation step in plasma preparation
- Benefit from a broad range of centrifugation conditions and no need for high-force centrifugation and cooling
- Possibilities for multianalyte workflows allowing detection of cfDNA, circulating tumor cell RNA and gDNA in a single blood sample

* See Voss et al (2021), PLOS ONE: https://doi.org/10.1371/journal. pone.0253401.

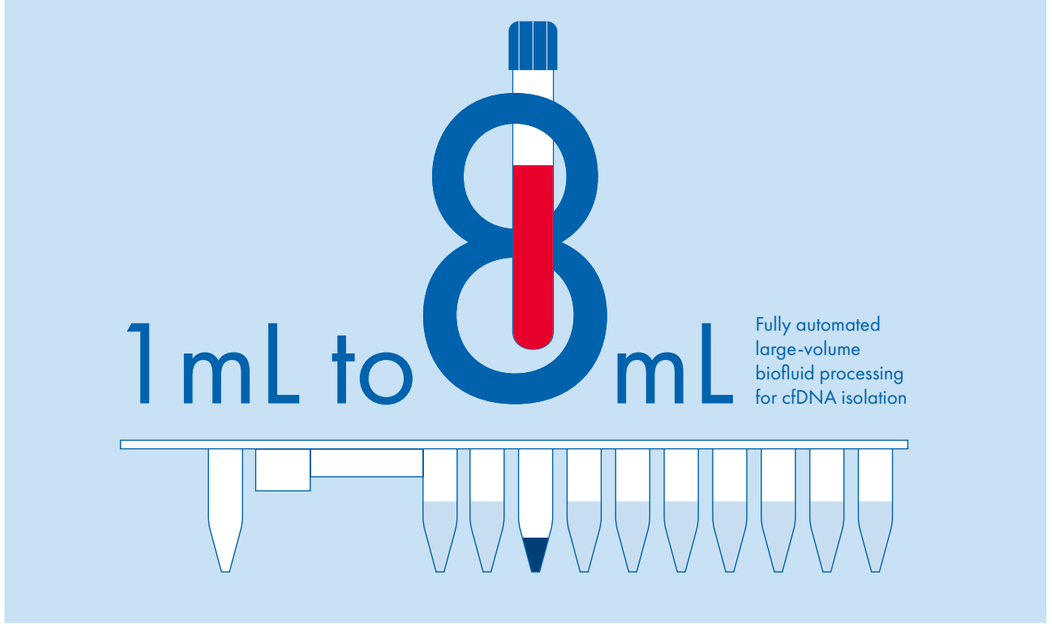
Get the Technical Note: A case study on multimodal analysis by PreAnalytiX.

Get the most from your precious sample

Manual and automated solutions

cfDNA preparation

Choose from our cfDNA extraction portfolio with unmatched flexibility, for high-yield, high-concentration cfDNA extraction: QIAamp MinElute ccfDNA and EZ1&2 ccfDNA Kits with scalable input volumes (Figure 3) and workflows that can be automated on QIAcube® Connect or EZ2® Connect. Or benefit from a fully validated, integrated and automated solution for direct processing of plasma within the tubes used to collect the blood samples with the QIAsymphony PAXgene Blood ccfDNA Kit.



Large-volume processing.
The EZ1&2 ccfDNA Kit processes 1–8 mL plasma.

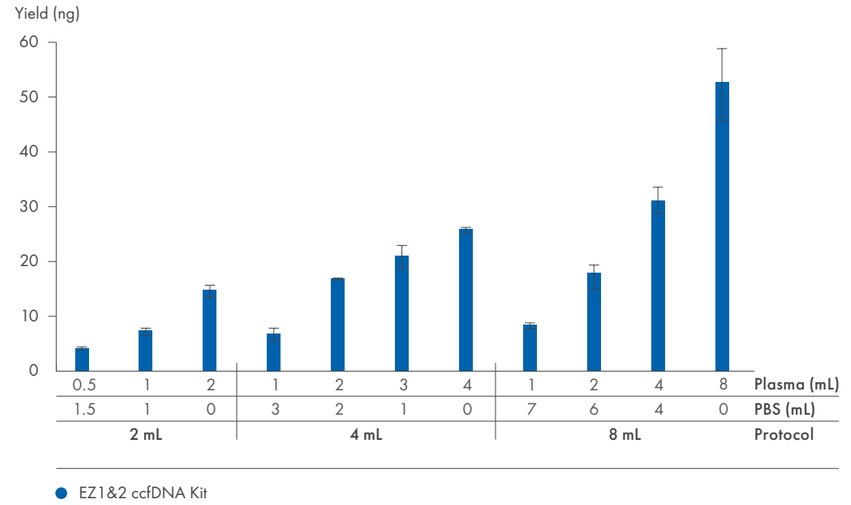


Figure 3. Flexible starting volumes.
Starting volumes were adjusted with PBS to allow processing with the standard protocols. Yields were determined using the QuantiPlex® Pro RGQ Kit (91 bp human fragment).

Technical Guide
From the collection tube, transport, centrifugation and cfDNA isolation to quality control, each step can be optimized with the help of your Technical Guide. See how your analytical processes can benefit immediately to accelerate your cancer research.

cfDNA analysis overview
Our range of products for cfDNA analysis is as broad as the applications that labs like yours are using. To find the set of products that are the ideal fit for your applications, from research to diagnostics, download this comprehensive aid to product selection.

Preparation of cell-free miRNA

The miRNeasy Serum/Plasma Advanced product line efficiently purifies total RNA, including miRNA, from serum and plasma samples. The phenol-free protocol is available in easy-to-automate MinElute spin column format or 96-well format for high-throughput projects. Analysis of RNA in and outside of vesicles is made possible by lysis of extracellular vesicles (Figure 4).

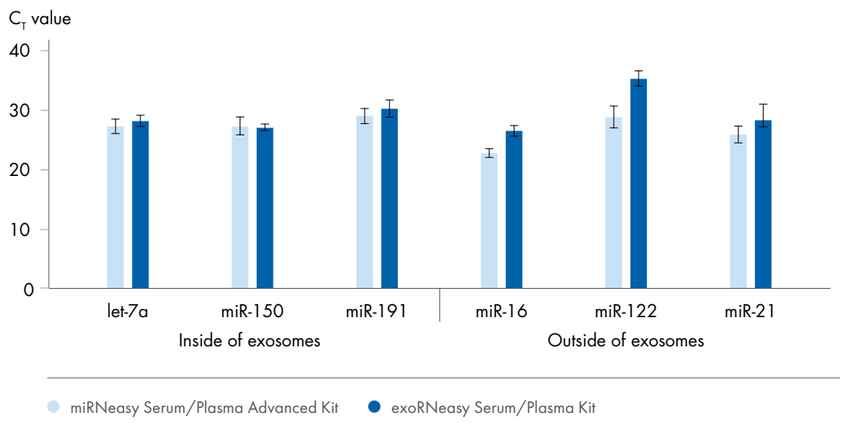


Figure 4. Detection of miRNAs preferentially found inside and outside of extracellular vesicles.
miRNA was isolated from 200 µL plasma from 20 different samples using the miRNeasy Serum/Plasma Advanced Kit. Five microliters of recovered RNA, in a total volume of 25 µL, was used for cDNA synthesis using the miScript® system, followed by PCR detection of 3 different miRNAs found to be primarily present inside or outside of extracellular vesicle, respectively. The miRNeasy Serum/Plasma Advanced Kit can detect miRNAs both inside and outside of vesicles.

Goodbye phenol, hello high yields!
Check out our phenol-free solutions for miRNA profiling.

Profiling biofluid miRNAs?
Maximize your miRNA sequencing and qPCR success from liquid biopsy samples, including serum/plasma, urine, CSF and exosomes.

Isolation of extracellular vesicles and related RNA

Explore the secrets hidden in exosomes. Enjoy spin column simplicity with ultracentrifugation quality – try the exoRNeasy and exoEasy Kits.

- Isolate extracellular vesicles in just 25 minutes
- Use high input volumes to get low abundance transcripts
- Separate vesicular vs. non-vesicular miRNA (Figure 5)

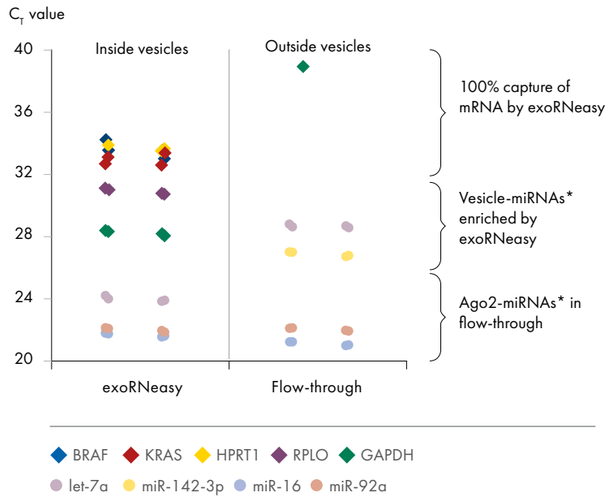


Figure 5. exoRNeasy technology captures all mRNA and vesicle-specific miRNAs in plasma.
RNA from pre-filtered plasma was isolated with the exoRNeasy Kit and the flow-through of the exoEasy column was used in direct lysis. Shown are raw CT values from RT-qPCR experiments with duplicate replicate isolations and duplicate qPCR reactions.

* Arroyo, J.D. et al. (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA **108**, 5003.

Molecular characterization of CTCs

The AdnaTest is a highly specific immunomagnetic cell selection system that combines an optimized antibody mixture with highly sensitive RT-PCR technology (Figure 6). The AdnaTest CTC Select Kit can be used manually or automated on the EZ2 Connect.

- Detection and molecular characterization of CTCs
- Highest CTC specificity and sensitivity

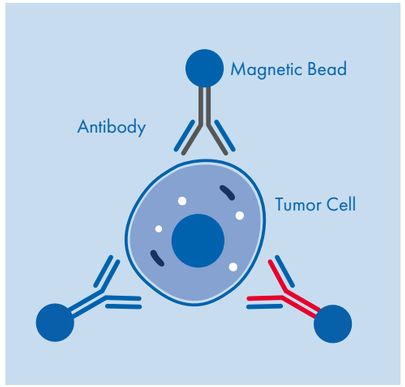
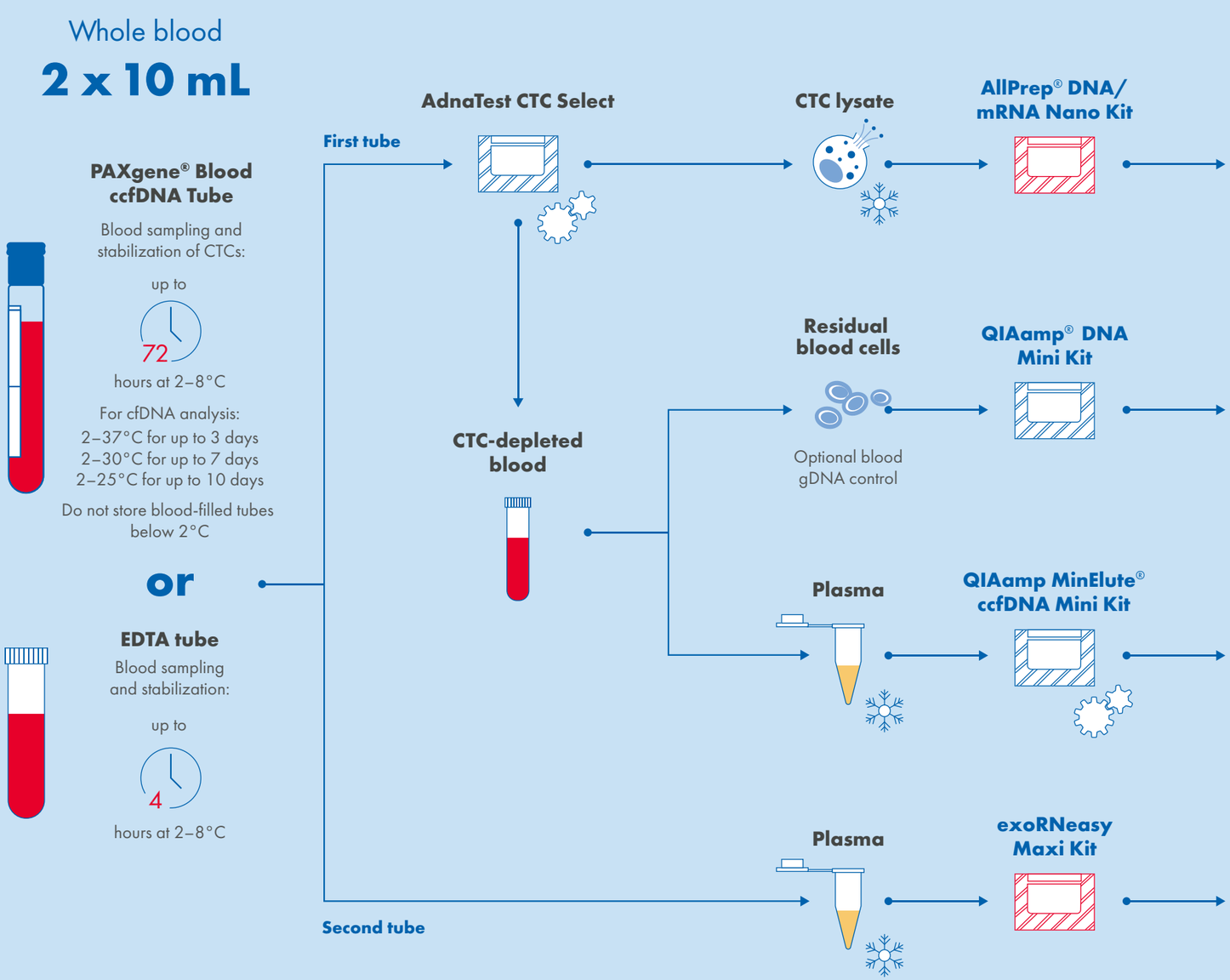


Figure 6. AdnaTest principle.
Tumor cell captured by three different antibodies ensuring highly specific isolation and detection of CTCs.

Quick-start workflow for multianalyte liquid biopsy research



Automation options available on EZ2 Connect or QIAsymphony

Storage possible

References

Keup C, et al. Cancers (Basel). 2020;12(5):1084.

Keup C, et al. Cell Mol Life Sci. 2020;77(3):497-509.

Keup C, et al. Cancers (Basel). 2019;11(2):238.

Keup C, et al. Clin Chem. 2018;64(7):1054-1062

Want to get started?

It is easier than you might think to get started with multianalyte experiments. Get in touch with us to identify the products you need for your analytes of interest.

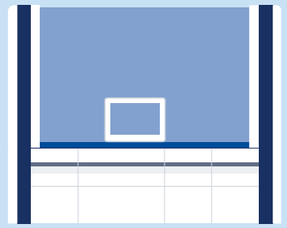
Contact us

Automate your liquid biopsy preparation

EZ2 Connect

Purify cfDNA from 1–24 samples full automated

- Boost reproducibility and convenience with prefilled reagent cartridges
- Achieve high sensitivity with fully automated large-volume processing of up to 8 mL
- Stay productive even outside the lab with QIAsphere® connectivity
- Minimize manual steps with onboard pipetting, heating and automated piercing of prefilled cartridge



QIAsymphony SP

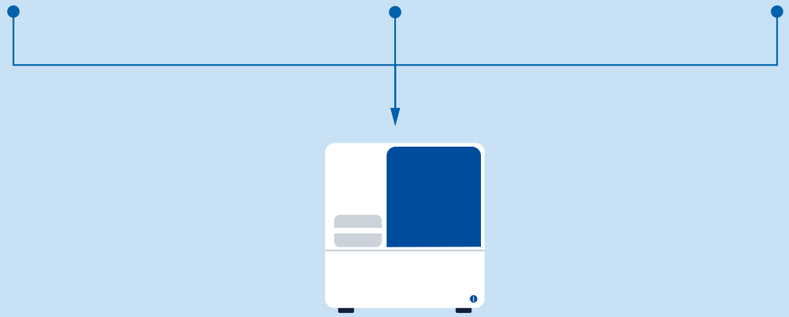
Fully automate your cfDNA sample preparation of 1–96 samples

- Continuously load your samples, with bar code reading for sample tracking
- Load ready-to-run reagent cartridges prefilled with all reagents required for purification
- Use validated protocols for 2 mL and 4 mL, customized protocols for large volumes (up to 10 mL) for molecular biology applications
- Import your sample lists and export your sample sheets
- Streamline workflow by direct processing of primary blood collection tube with a primary tube handling option with same high cfDNA yields
- Flexible plasma input

QIAcube Connect

Automate over 80 QIAGEN kits with over 140 standard protocols for DNA, RNA and protein sample processing

- Process up to 10 mL input volume
- Analyze high concentrations due to elution volumes down to 20 µL
- Increase your efficiency with QIAsphere* and monitor your runs remotely by using the QIAsphere App



QIAxcel Connect System

Fully automate quality control of up to 96 samples per run

- Perform quality control of your cfDNA samples and NGS libraries with a limit of detection of 5 pg/µL
- Collect high-resolution data by DNA capillary electrophoresis
- Receive your results in real time
- Eliminate tedious gel or consumable preparation by using ready-to-run gel cartridges
- Stay productive outside the lab with the option of the QIAsphere system

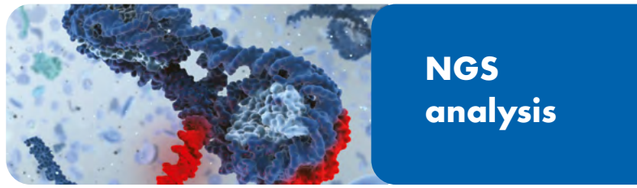
* Certain features require a subscription.

Discover biomarkers in any liquid biopsies

QIAseq Targeted cfDNA Ultra Panels

The QIAseq Targeted cfDNA Ultra enables streamlined Sample to Insight, targeted NGS of cfDNA. This highly optimized, automation-friendly solution facilitates ultrasensitive variant detection down to 0.1% by using integrated unique molecular indices (UMIs; Figure 7) and high-fidelity chemistry from biofluids within 8 hours and is coupled with an error-correction data analysis.

A single QIAseq Targeted cfDNA Ultra sequencing reaction requires only 5–80 ng of cfDNA template.



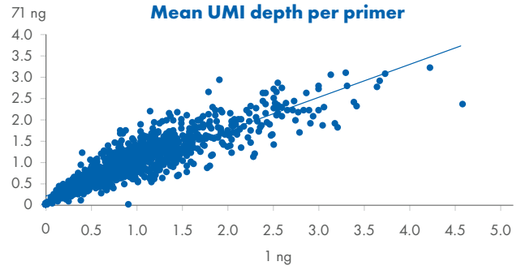


Figure 7. Consistent UMI depth between low and high cfDNA inputs. Although equivalent UMI depth was achieved at 1 ng cfDNA when compared to 71 ng, we recommend at least 30 ng cfDNA to detect variants below 0.2% frequency.

QIAseq library preparation

For new biomarker discoveries or to search for aneuploidies from any cell-free DNA sample, the QIAseq Ultralow Input Library Kit provides highly efficient library preparation to ensure maximum sample conversion (Figure 8) and sensitive variant detection – for whole genome or exome sequencing applications.

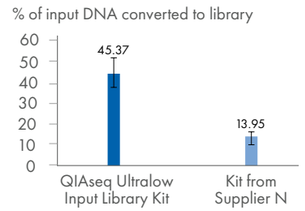


Figure 8. Superior conversion rate of cfDNA to NGS library. The average calculated conversion rate of the replicate samples is displayed. QIAseq Ultralow Input Library Kit shows significantly higher conversion rates.

QIAseq miRNA library preparation

QIAseq miRNA sequencing kits deliver high-quality data by using UMIs and a simple gel-free workflow. The QIAseq miRNA Library Kit achieves greater sequencing efficiency by eliminating adapter dimers and unwanted RNA species, resulting in the most efficient use of your sequencing instrument for miRNA, piRNA and small RNA discovery (Figure 9).

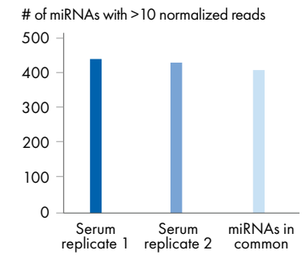


Figure 9. High yields. The QIAseq miRNA Sequencing Kit has been designed to enhance yields from biofluids such as serum.

Validate liquid biopsy biomarkers with dPCR

dPCR analysis

The use of digital PCR (dPCR) in liquid biopsy is underscored by its ability to analyze the cfDNA, ctDNA, CTCs and miRNAs of interest present at low levels or highly fragmented or found in a complex background of other components.

QIAcuity dPCR is performed in a nanoplate containing thousands of stable partitions, providing a user-friendly workflow with a time to result of about two hours and a precise and sensitive method to characterize relevant biomarkers. A specific benefit of using the QIAcuity Nanoplate 26K is that it increases the limit of detection (LOD) and minimizes the subsampling error. It also saves precious samples as replicates are not required. High-volume sample processing on EZ2 Connect combined with mutation analysis using dPCR LNA Mutation Assays on QIAcuity results in accurate detection of mutation copies (Figure 10).

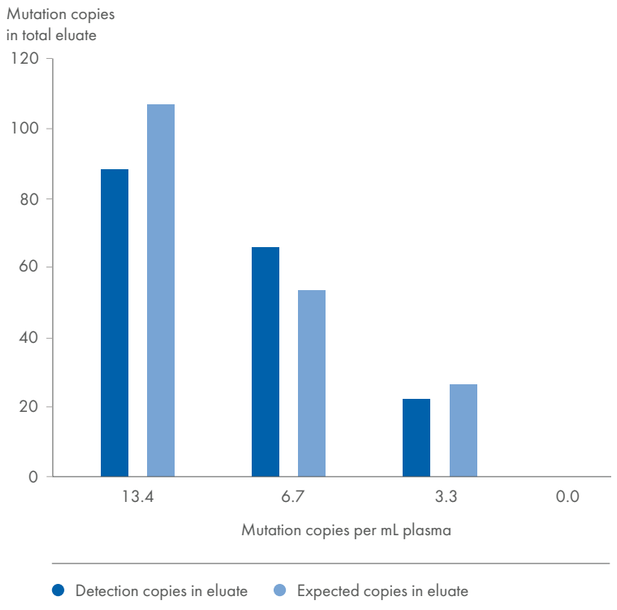


Figure 10. Good correlation between spiked copies and dPCR results.
Expected cfDNA copy numbers correlate well with detected copy numbers using the QIAcuity dPCR system for liquid biopsies. Blood from healthy donors using PAXgene ccfDNA Tubes isolated with the EZ2 Connect and cfDNA from human cell lines with a PIK3CA p.H1047R mutation and defined mutation rate was spiked into plasma as reference. A dPCR LNA PIK3CA p.H1047R Mutation Assay was run on the QIAcuity in a Nanoplate 26K. The expected copies in the eluate were calculated based on plasma input (light blue column). The dark blue column represents the detected copies in the eluate. The X-axis depicts the number of spiked-in mutation copies per mL plasma decreasing from 13.4 copies to zero.

Explore disease-specific biomarker signatures with qPCR

Simultaneously profile mRNA, miRNA and lncRNA

The study of RNA has evolved from the simplicity of the central dogma of molecular biology. And there are multiple known noncoding RNA species that directly regulate gene expression. To truly understand gene expression, exploring regulatory RNA, such as miRNA and lncRNA, is key (Figure 11).

- miRCURY LNA PCR Panels
- QuantiNova LNA PCR Assays and Panels
- QuantiNova LNA Probe PCR Assays and Panels

Solutions for mRNA quantification

The QuantiNova family of qPCR kits combines top-notch performance with various in-process control features to ensure unbiased, reproducible results – even with minor changes in transcript levels. Process and analysis errors are reduced with:

qPCR analysis

- Internal Control RNA
- Visual pipetting control
- gDNA removal
- Room temperature setup

While the QuantiNova Multiplex PCR and RT-PCR Kits provide the maximum information from a single PCR run, a complete kit portfolio for 1- or 2-step RT-PCR covers any qPCR need you may have for screening or confirmation – delivering instant success at the first attempt.

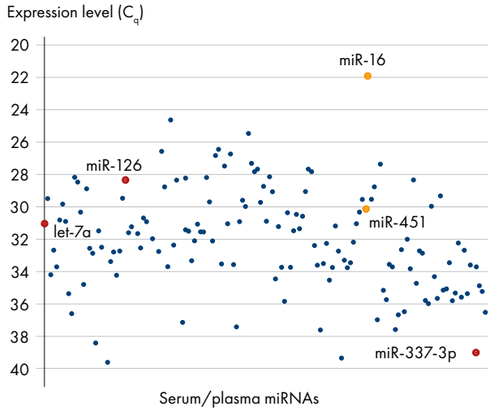


Figure 11. Complete miRNA serum profile.
The miRCURY LNA miRNA Serum/Plasma Focus PCR Panels are designed for profiling 175 human miRNAs commonly found in serum and plasma. Real-time PCR was performed using triplicate RT reactions on total RNA purified from serum. Average expression levels in Cq (quantification cycle) values for miRNAs profiled with the Serum/Plasma Focus miRNA panel are shown. hsa-miR-16, which is known to be highly expressed in serum/plasma, and miR-451 are considered potential indicators of hemolysis and blood cell contamination in serum/plasma (shown in orange). Other interesting plasma miRNAs are highlighted in red.

Concentrate on insights, not data – bioinformatics solutions

Data analysis and interpretation

Variant identification and interpretation of cfDNA

QIAGEN Bioinformatics Advanced Testing Solution, featuring CLC Genomics Workbench and QCI Interpret, combines state-of the-art data analysis and data interpretation into a single offering. Optimized and streamlined end-to-end workflows enable high sensitivity in detection, filtering and interpretation of known and potential new cancer driver variants down to 1% (or lower) allelic fractions.

Sequencing of RNA from extracellular vesicles

The RNA-seq Analysis Portal enables you to start from FASTQ and align, analyze, QC, normalize and determine differential expression. Now you can identify the biology associated with differentially expressed genes and isoforms of varying expressions without being a bioinformatician.

Understand complex ‘omics data with IPA

Ingenuity Pathway Analysis (IPA) is a powerful analysis and search tool that uncovers the significance of ‘omics data and identifies new targets or candidate biomarkers within the context of biological systems. IPA has broadly been adopted by the life science research community and is cited in thousands of articles for the analysis, integration and interpretation of data derived from ‘omics experiments, such as RNA-seq, small RNA-seq, microarrays including miRNA and SNP, metabolomics, proteomics and small-scale experiments.

Complete workflow design

GeneGlobe Design & Analysis Hub

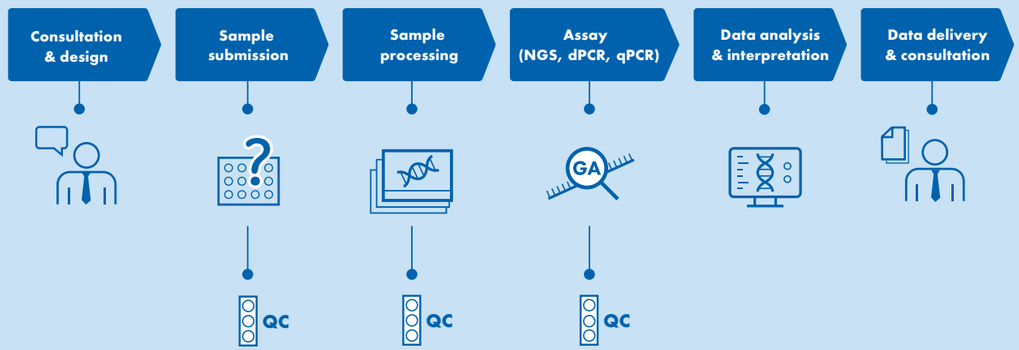
- Quickly and easily explore targets in their scientific context and build gene lists
- Find and customize the right products to study those targets
- Analyze the data and plan your follow-up studies based on insights gained from embedded PCR and NGS analysis pipelines
- Includes the RNA-seq Analysis Portal (RAP) for all QIAseq miRNA-seq and RNA-seq Kits

Don’t let limited resources hold you back

Get support from QIAGEN Genomic Services

Partnering with Genomic Services is a straightforward and reliable way to extend your in-house resources and ensure you get high-quality data. To help you make impactful discoveries, our dedicated team is happy to help with the time-consuming parts of your project or support you through the entire experimental journey. The Genomic Services lab provides all-in-one solutions, from proven sample preparation to robust data analysis and interpretation.

How our Sample to Insight solution works



Genomic Services solutions for biofluids

Profiling of miRNAs in biofluid samples holds great promise due to their potential use as minimally invasive biomarkers for various diseases. Still, a number of challenges are associated with miRNA profiling. Compared to tissues, cells or whole blood, the concentration of miRNAs in cell-free biofluids is very low, making their detection and quantification difficult. QIAGEN Genomic Services overcomes these limitations by combining our innovative QIAseq miRNA-seq technology with decades of technical expertise with biofluid samples. Extend your in-house resources with the expertise and high-quality services you can expect from us.

Genomic Services all-in-one biofluids miRNA seq service offers:

- End-to-end service: we take care of every step, from sample preparation to data analysis
- qPCR-based quality controls: locked nucleic acid (LNA) RNA spike-in controls to monitor RNA extraction efficiency, miRNA content, inhibition and hemolysis
- Highly efficient library preparation: optimized protocols for biofluids with limited RNA content
- Sequencing quality control: evaluating data reproducibility and linearity using a comprehensive set of 52 RNA spike-ins, spanning a wide range of concentrations
- Ready-to-publish data: we deliver comprehensive reports and data packages and provide guidance on the next steps

Visit **www.qiagen.com/liquidbiopsy** today to find out more about liquid biopsy analysis products!

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