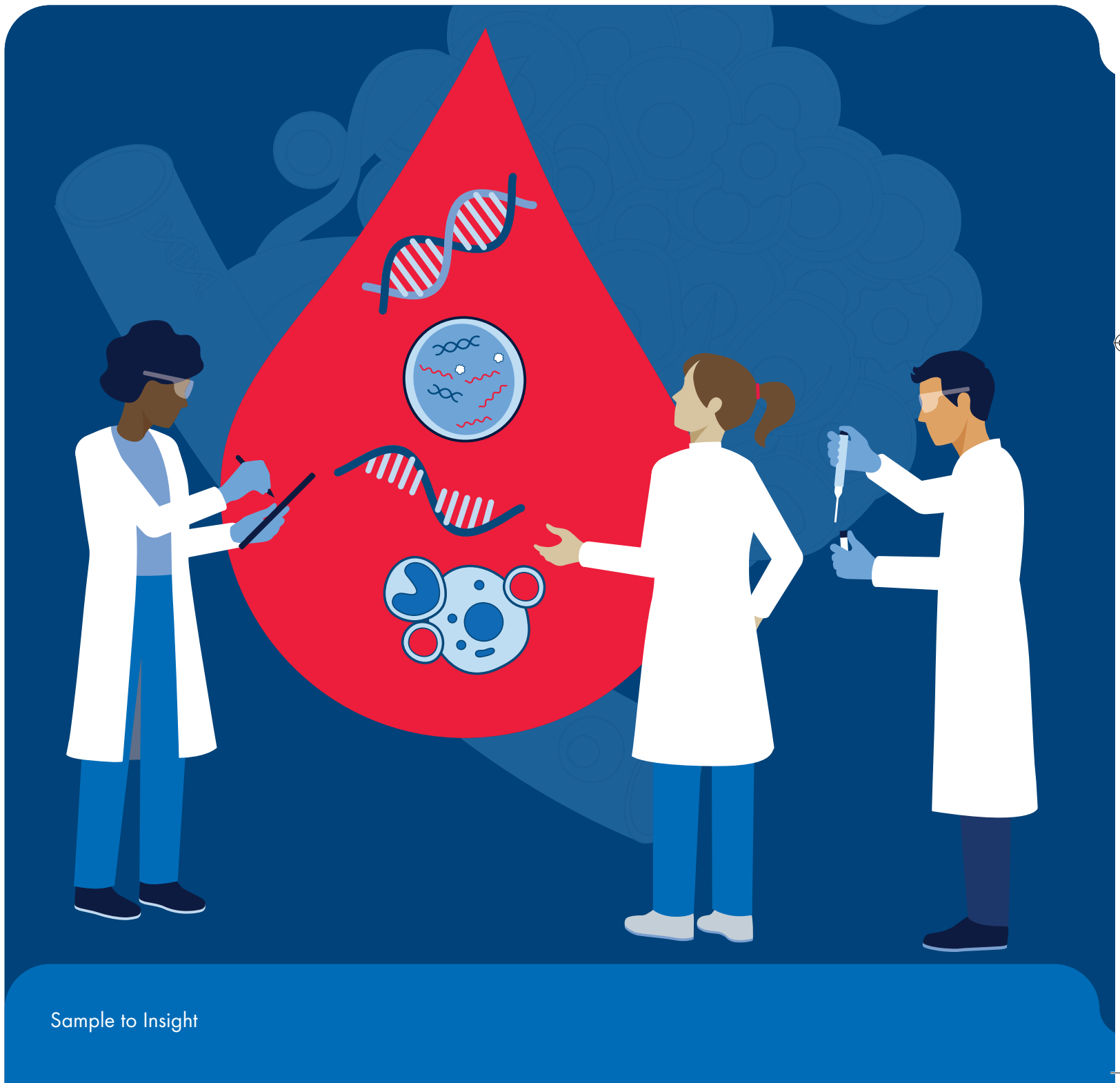




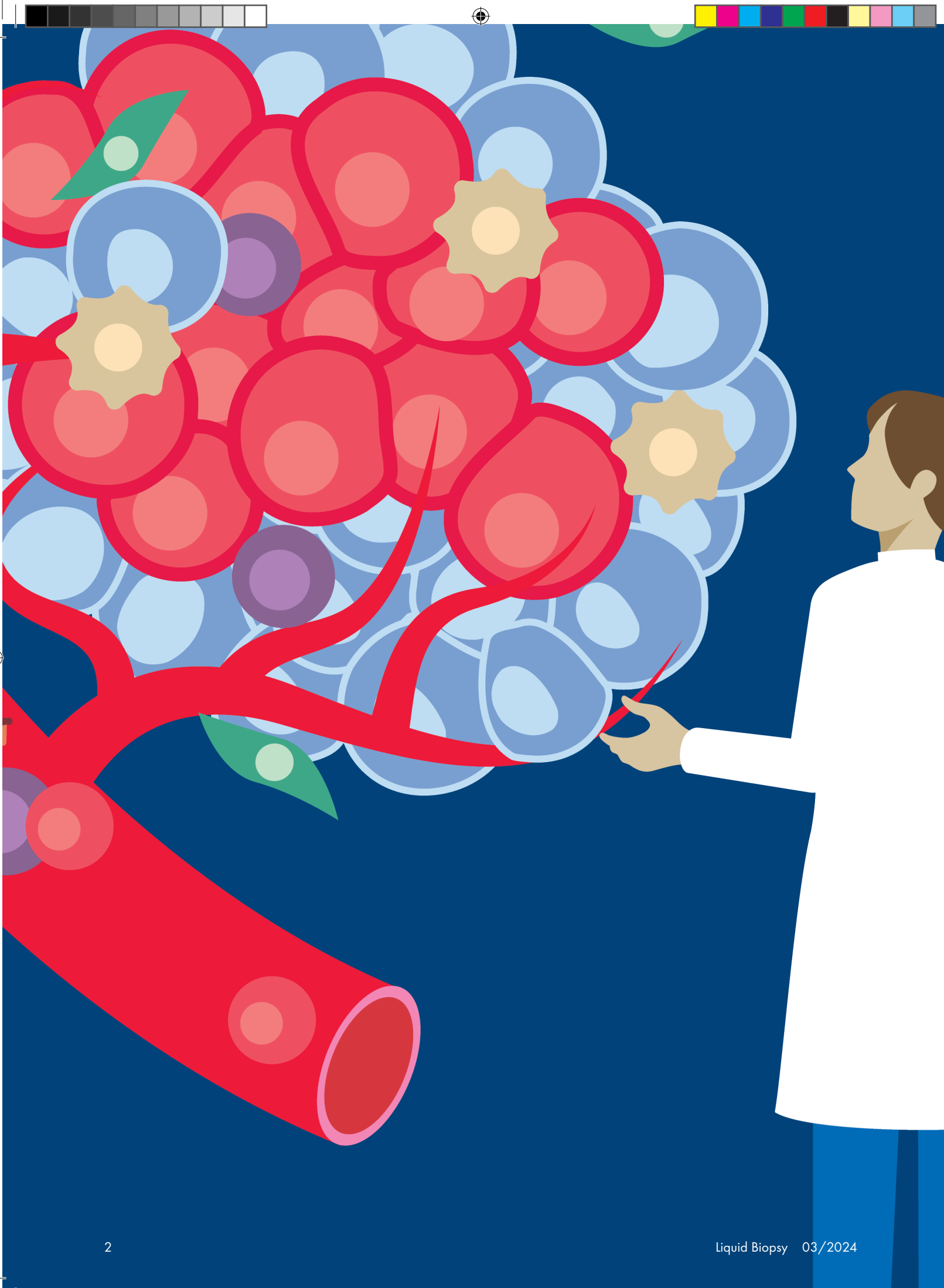
Liquid Biopsy

Accelerate Your Circulating
Biomarker Research



Sample to Insight





Cross the bridge from discovery to insight

Liquid biopsy is a non-invasive approach that detects circulating tumor cells (CTCs) and molecular biomarkers in blood, urine and other body fluids. It is particularly useful when:

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

Despite its potential, challenges such as low sensitivity and specificity of detection methods, lack of standardized protocols and poor scalability have slowed widespread adoption. Our comprehensive liquid biopsy workflow addresses many of these challenges, enabling you to go seamlessly from discover to insight.

Liquid biopsy begins with reliable sample collection and processing. Our manual and automated sample preparation methods cover a wide range of liquid biopsy analytes, including circulating cell-free nucleic acids, exosomes and CTCs.

The QIAGEN® next-generation sequencing (NGS) library workflows ensure the most accurate and reproducible analysis for DNA, RNA, and miRNA as well as multimodal analysis of DNA and RNA.



The QIAcuity® 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.

Together with QIAGEN Digital Insights solutions for data analysis and interpretation, our end-to-end workflow for liquid biopsy analysis enables high sensitivity in detecting, filtering and interpreting variants.





qPCR analysis / dPCR analysis

Data analysis

Interpretation

- 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

- Identification and absolute quantification of mutations
- Biomarker validation
- Minimal residual disease (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
- dPCR PanCancer Kits

miRNA biomarker profiling

- miRCURY LNA Digital PCR Assays

Gene expression analysis

- QuantiNova® LNA PCR Assays for Digital PCR

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

- QIAGEN CLC Genomics Workbench
- GeneGlobe® Design & Analysis Hub

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

- QIAGEN Clinical Insight (QCI®) Interpret
- QIAGEN Ingenuity® Pathway Analysis (IPA®)



QIAGEN 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

* The PAXgene Blood ccfDNA Tube (IVD) is distributed by BD and QIAGEN and their distributors are not available in certain countries including the US. Please visit www.preanalytix.com or contact your local supplier for more details and product availability.

Standardize your preanalytical workflow to ensure 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.

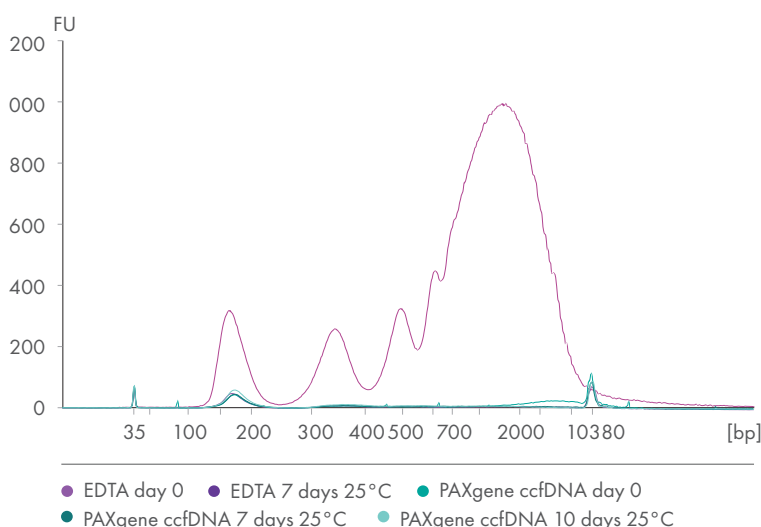


Figure 1. PAXgene Blood ccfDNA stabilization helps prevent release of gDNA into plasma.

Whole blood was stored in EDTA tubes or PAXgene Blood ccfDNA Tubes. 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 showed a cfDNA profile comparable to day 0.

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 modifications: Compatible with different downstream assays, including methylation-based assays
- Maximize assay sensitivity by avoiding missing critical mutations†

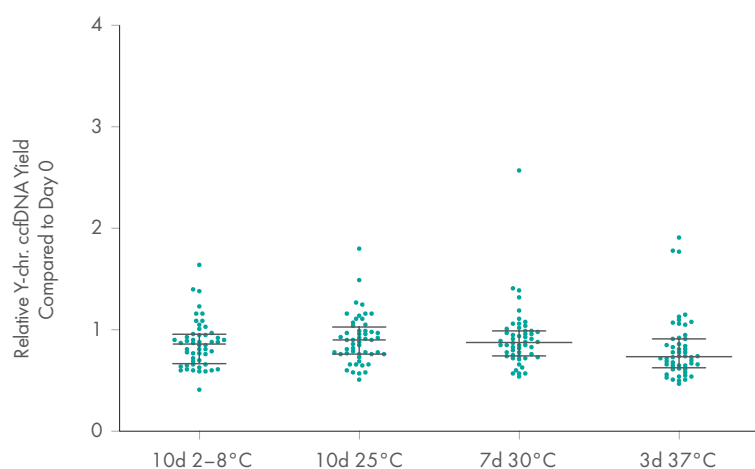


Figure 2. Preservation of cfDNA yield in plasma from blood samples stored in PAXgene Blood ccfDNA Tubes
Change in Y-chromosomal male plasma cfDNA yield after whole blood sample storage in comparison to plasma separated within 2 hours of blood collection (Day 0). Blood was drawn from 10 male and 26 female 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 QIAasymphony PAXgene Blood ccfDNA Kit on the QIAasymphony 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
- Benefit from a broad range of centrifugation conditions and no need for high-force centrifugation and cooling

Streamline preanalytical workflow in conjunction with validated purification kits

- Eliminate a second centrifugation step in plasma preparation by processing primary blood collection tubes on QIAasymphony SP
- Benefit from cfDNA workflow CE marked for In Vitro Diagnostic Use according to Regulation (EU) 2017/746 on In Vitro Diagnostic Medical Devices *
- Possibilities for multianalyte workflows allowing detection of cfDNA, circulating RNA (EV-derived), CTC nucleic acids and gDNA in a single blood draw (in research applications only) *

* The PAXgene Blood ccfDNA Tube (IVD) is distributed by BD and QIAGEN and their distributors are not available in certain countries including the US. Please visit www.preanalytix.com or contact your local supplier for more details and product availability.

† Voss T, Ullius A, Schönborn M, Oelmüller U. Sensitivity assessment of workflows detecting rare circulating cell-free DNA targets: A study design proposal. *PLoS One*. 2021;16(7):e0253401.

➞ Get the Technical Note for further application data on multianalyte research by PreAnalytiX.



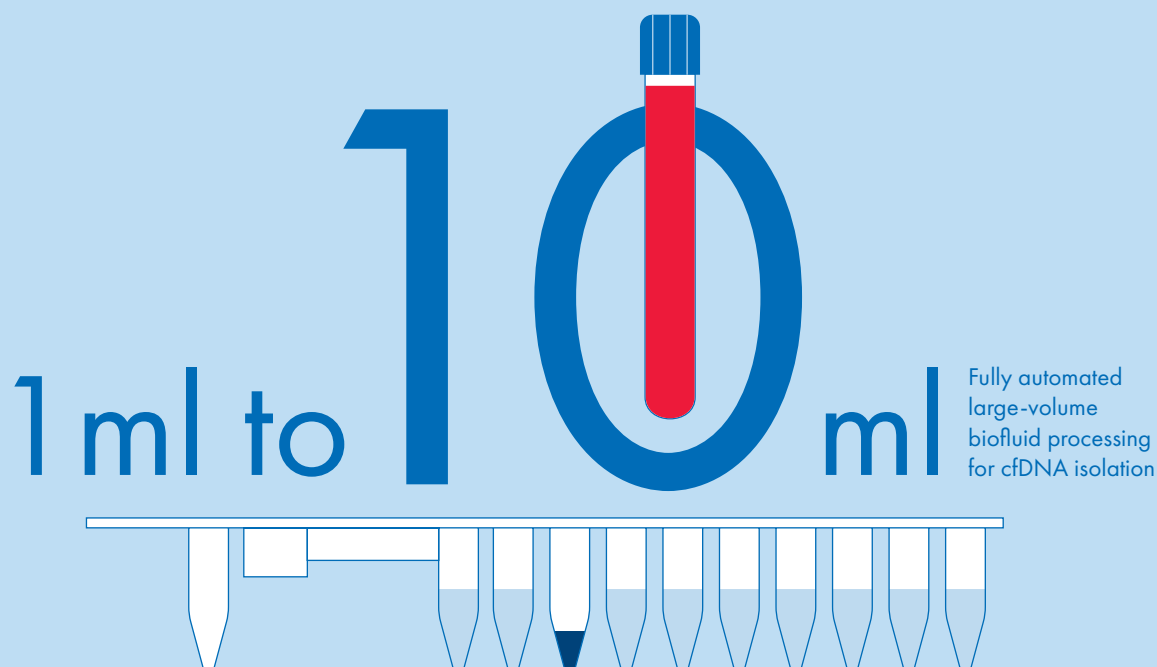
Get the most from your precious sample

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 solution with automated direct plasma processing of centrifuged primary PAXgene Blood ccfDNA Tubes with the QIAasymphony PAXgene Blood ccfDNA Kit.



Large-volume processing.

The EZ1&2 ccfDNA Kit processes 1–10 mL plasma.

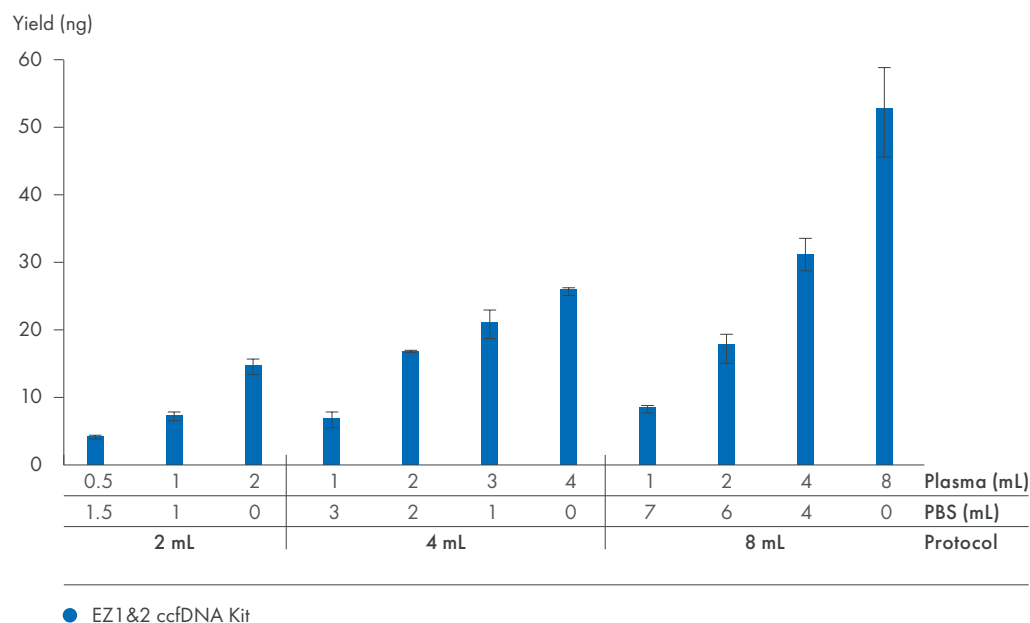


Figure 3. Flexible starting volumes.

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).



Biomarker profiling from cfDNA

From sample collection to cfDNA isolation and quality control, each step can be optimized with the help of this Technical Guide. Download now.



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 right set of products for your application, 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).

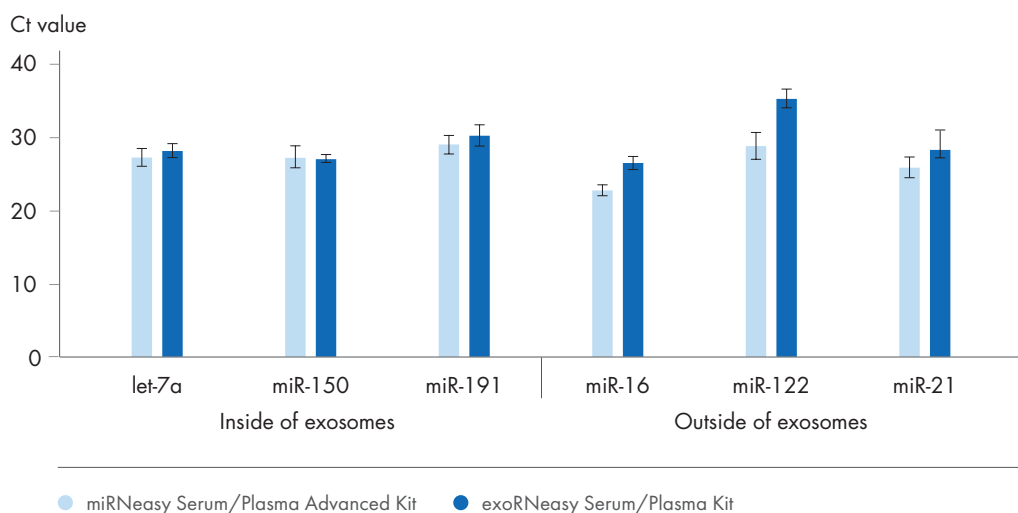


Figure 4. Detection of miRNAs preferentially found inside and outside of extracellular vesicles.

miRNA was isolated from 200 μ L plasma from 20 different samples using the miRNeasy Serum/Plasma Advanced Kit. Five microliters of recovered RNA, in a total volume of 25 μ L, 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)

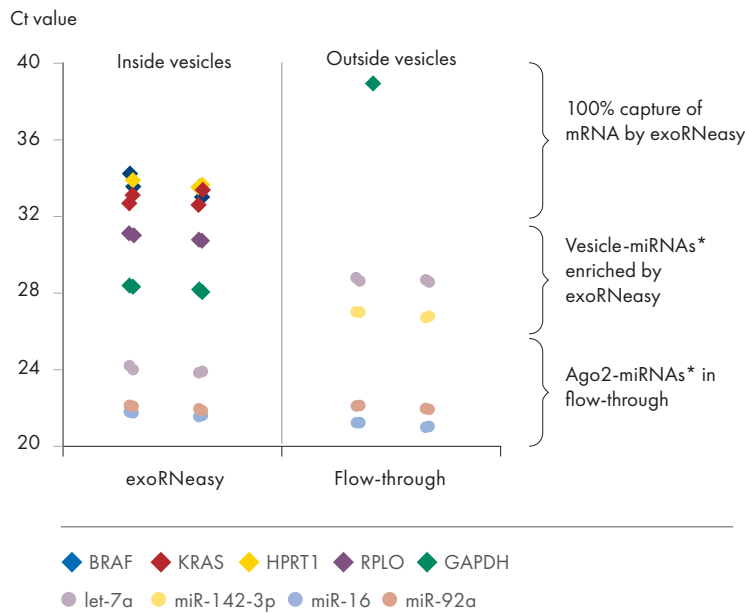


Figure 5. exoRNeasy technology captures all mRNA and vesicle-specific miRNAs in plasma.

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 JD, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A.* 2011;108(12):5003–5008.

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). AdnaTest uses a two-step magnetic bead process to capture tumor cells and their mRNA from a blood sample. cDNA obtained through reverse transcription is then analyzed to identify specific markers linked to cancer. Its unique design allows customized analysis of CTCs, including downstream NGS applications. 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

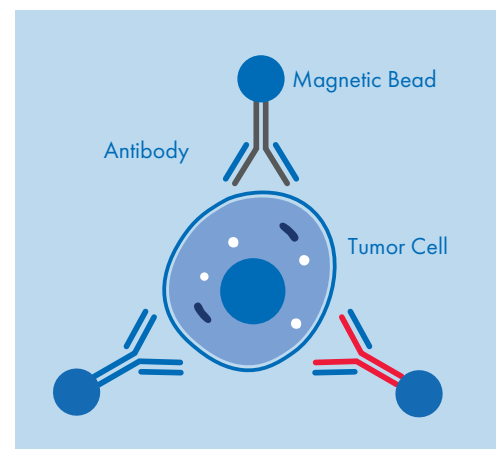


Figure 6. AdnaTest principle.

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

Quick-start workflow for multianalyte I

Whole blood
2 x 10 mL

PAXgene Blood ccfDNA Tube

Blood sampling and stabilization of CTCs:

up to



hours at 2–8°C

For ccfDNA analysis:

2–37°C for up to 3 days

2–30°C for up to 7 days

2–25°C for up to 10 days

Do not store blood-filled tubes below 2°C

or

EDTA tube

Blood sampling and stabilization:

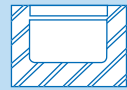
up to



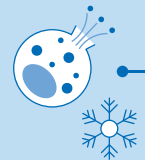
hours at 2–8°C

AdnaTest CTC Select

First tube



CTC lysate



CTC-depleted blood

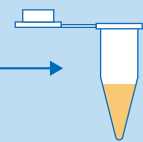


Residual blood cells

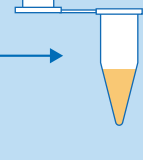


Optional blood gDNA control

Plasma



Plasma



Second tube

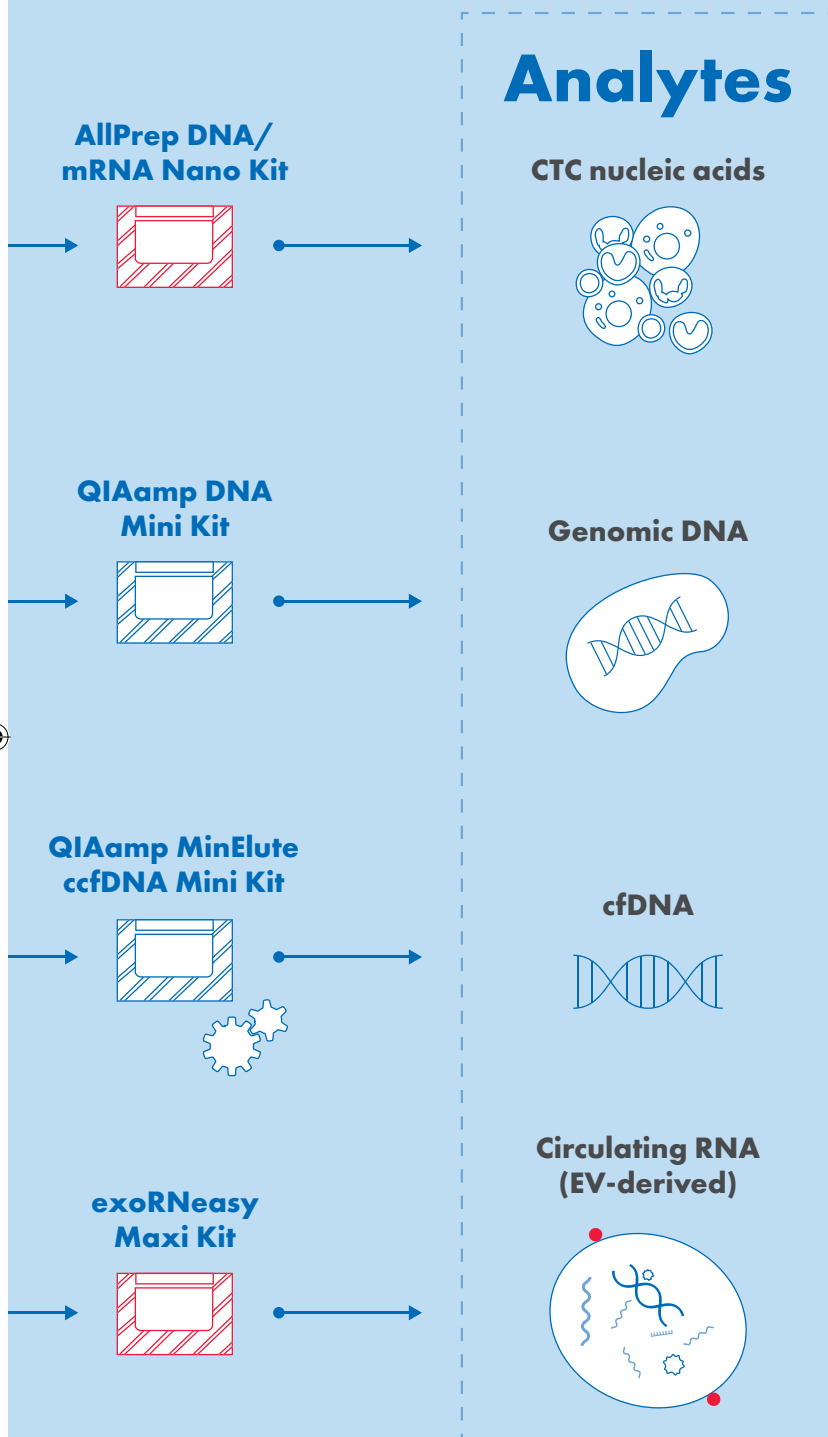


Automation options available on EZ2 Connect or QIAasympy



Storage possible

liquid biopsy research



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.
Get in touch with us to identify
the products you need for your
analytes of interest.

Contact us



Automate your liquid biopsy preparation

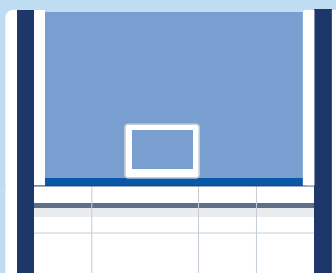
Process up to 10 mL input volume using any of these instruments



EZ2 Connect

Automated processing of 1–24 samples per run

- Boost reproducibility and convenience with prefilled reagent cartridges
- Achieve high sensitivity with fully automated large-volume processing of up to 10 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

Automated processing of 1–96 samples per run

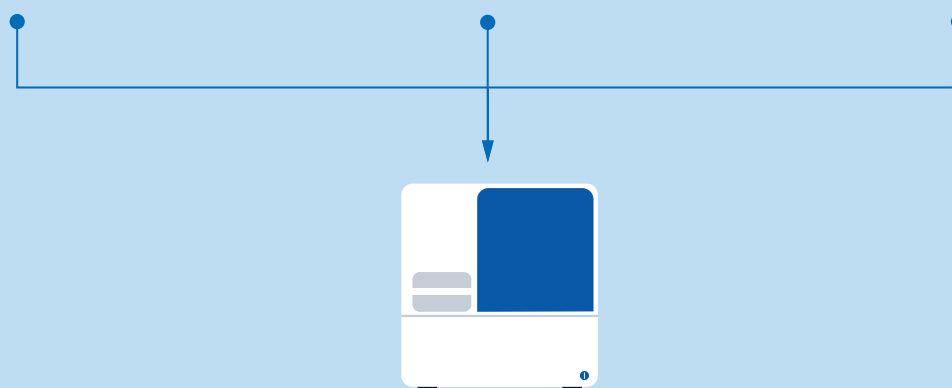
- Continuously load your samples, with bar code reading for sample tracking
- Load ready-to-run reagent cartridges prefilled with all reagents required for purification
- Use validated protocols for 2 mL and 4 mL, and customized protocols for higher starting volumes for molecular biology applications
- Import your sample lists and export your sample sheets
- Streamline workflow by direct processing of centrifuged primary PAXgene Blood ccfDNA Tubes with same high cfDNA yields
- Flexible plasma input



QIAcube Connect

Automated processing of 1–12 samples per run

- 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 automated 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

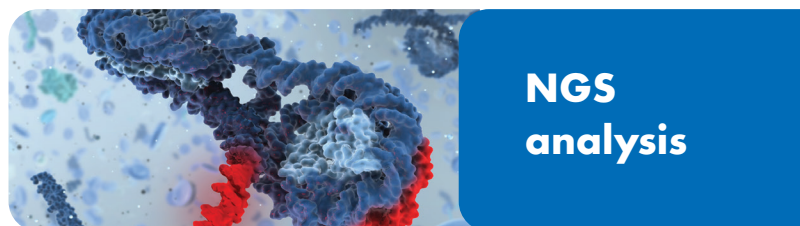
* Certain features require a subscription.

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.



**NGS
analysis**

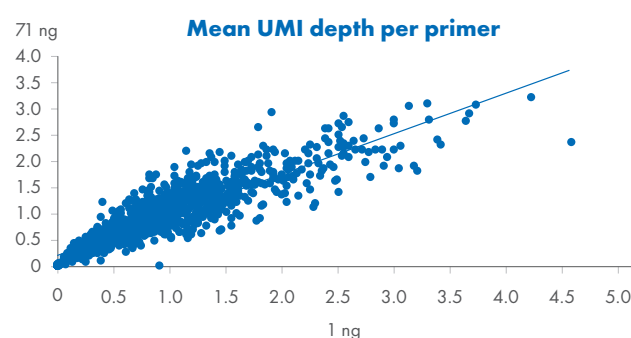


Figure 7. Consistent UMI depth between low and high cfDNA inputs.
The QIAseq Targeted cfDNA Ultra Panels achieved equivalent UMI depth at 1 ng cfDNA when compared to 71 ng. However, we recommend at least 30 ng cfDNA to detect variants below 0.2% frequency.

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.

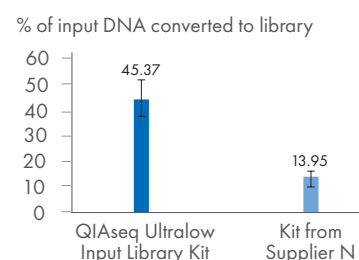


Figure 8. Superior conversion rate of cfDNA to NGS library.

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).

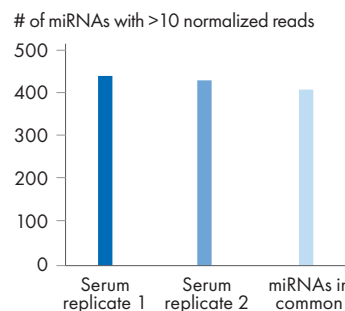


Figure 9. High yields.

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.

Table 1: QIAcuity dPCR kits and assays for liquid biopsy analysis

Application	QIAcuity dPCR kits and assays
Mutation analysis	dPCR LNA Mutation Assays
	dPCR PanCancer Kits
CNV analysis	dPCR CNV Probe Assays
miRNA biomarker profiling	miRCURY LNA Digital PCR Assays
Gene expression analysis	QuantiNova LNA PCR Assays for Digital PCR

QIAcuity dPCR is performed in a nanoplate containing thousands of stable partitions, enabling a user-friendly two-hour workflow for precise biomarker characterization. A specific benefit of using the QIAcuity Nanoplate 26k is that it maximizes resolution and saves precious samples, as replicates are not required.

High-volume sample processing on EZ2 Connect can be combined with dPCR LNA Mutation Assays

on QIAcuity for accurate mutational analysis. dPCR is a highly sensitive method for analyzing key mutations in cancer-associated genes in more detail, thus advancing clinical and translational research. The dPCR PanCancer Kits on QIAcuity enable parallel detection of hallmark mutations – 8 *BRAF* V600 mutations and 23 *EGFR* exon 19 deletions – in a single dPCR reaction (Figure 10).

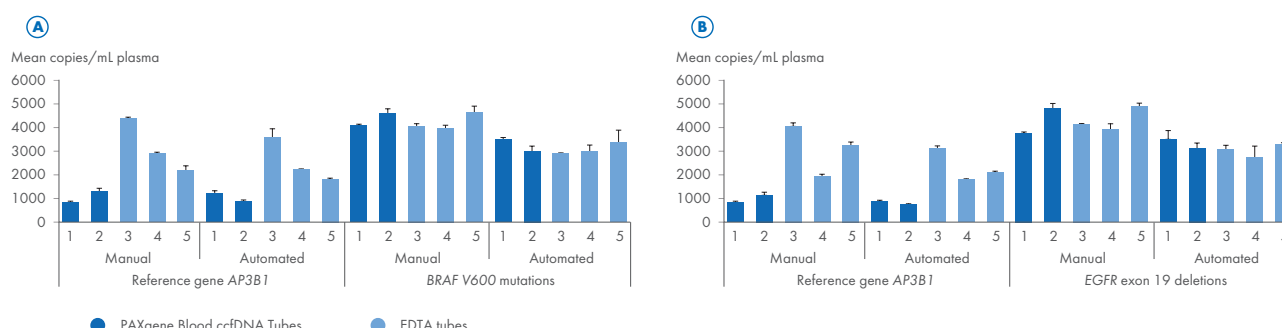


Figure 10. Detection of *BRAF* V600 mutations (A) and *EGFR* exon 19 deletions (B) in cfDNA extracted from blood plasma using the dPCR PanCancer Kit on the QIAcuity platform.

DNA was extracted – both manually and automated – from freshly prepared plasma using blood from five healthy donors. Manual extraction was done using QIAamp Circulating Nucleic Acid Kit and automated extractions using EZ1&2 ccfDNA Kit on EZ2 Connect. Samples 1 and 2 were prepared using blood collected in PAXgene Blood ccfDNA Tubes and samples 3–5 using EDTA tubes. To simulate the presence of mutated DNA content, 6000 copies of synthetic DNA (GBlocks) were spiked into 1 mL plasma prior to the DNA extraction process. In the subsequent dPCR reactions, 2 μ L of the eluate served as input. This quantity represented 334 μ L of plasma from the EZ2 preparation. The amount of cfDNA loaded into the dPCR reaction ranged from 0.4–2.2 ng depending on the donor. The averages of two replicates are shown with standard deviations.

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:



- 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.

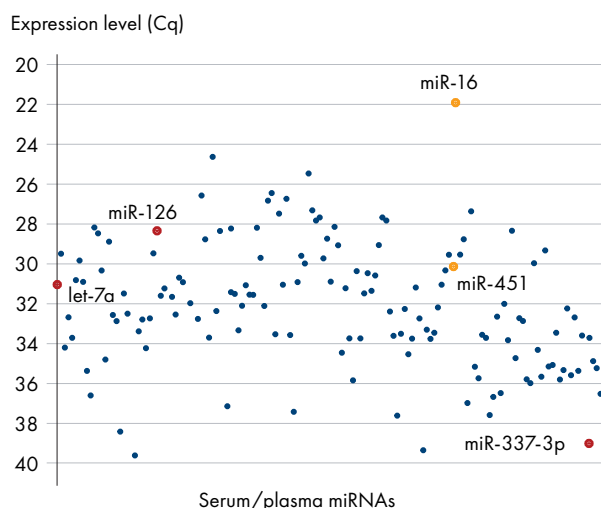


Figure 11. Complete miRNA serum profile.

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 when combined with QIAseq Targeted cfDNA Ultra Panels) 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 QIAGEN IPA

QIAGEN 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

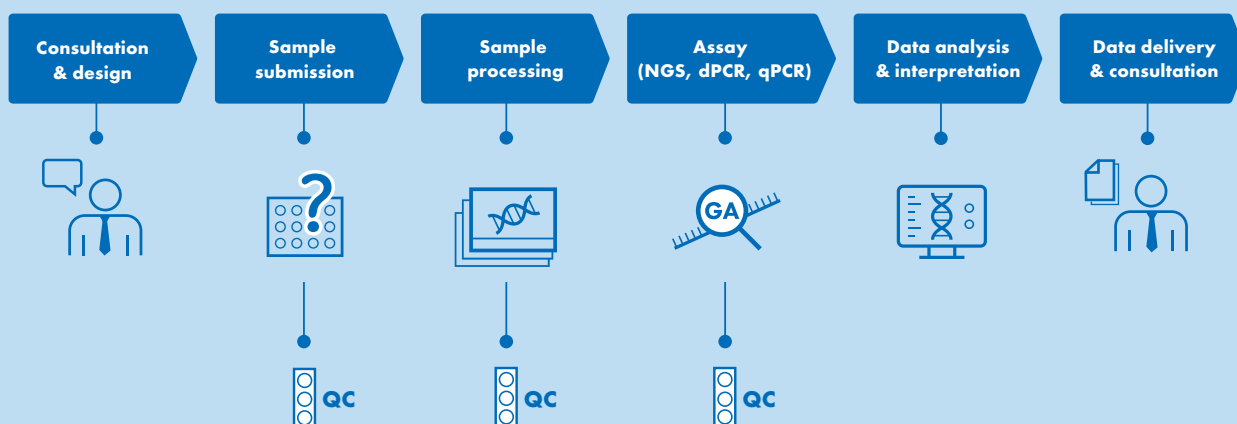
Don't let limited resources hold you back

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

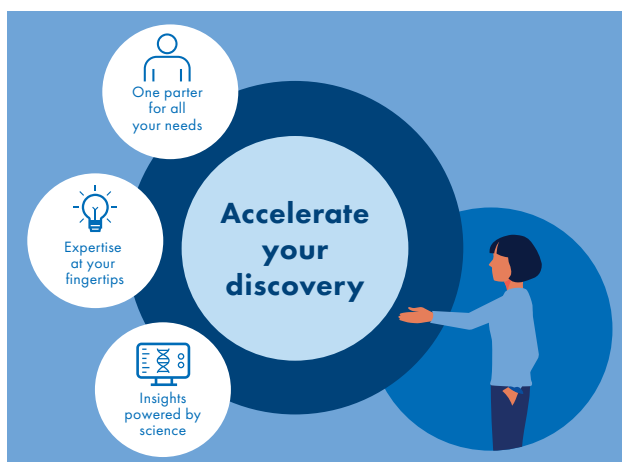


Genomic Services solutions for biofluids

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



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