Paving the way for liquid biopsy

Integrated cell-free DNA workflow solutions

Integrated, complete cell-free DNA workflow solutions for any needs:

- cfDNA analysis from sample collection to actionable insights
- cfDNA preparation for varying application needs
- Mutation or methylation analysis for variant detection or screening applications
- Next-generation sequencing and digital PCR technologies to accelerate your research

Sample to Insight

Sample to Insight® cell-free DNA analysis

Comprehensive workflow solutions starting with blood collection and stabilization along the molecular analysis workflow from dedicated cfDNA preparation to integrated data analysis solutions for various applications by next-generation sequencing or digital PCR, scalable to your sample volume, throughput or outsourcing needs.

Sample collection and stabilization

Single tube blood collection, stabilization, transport and storage for standardized preanalytical processing with an integrated solution.

- PAXgene® Blood ccfDNA Tubes

Cell-free DNA preparation

Complete portfolio of manual and low, medium and high-throughput automation solutions for cfDNA preparation optimized to achieve high sensitivity in downstream applications.

- EZ2® Connect
- QIAsymphony® SP
- QIAcube® Connect

Manual cfDNA preparation:

- QIAamp® MinElute® ccfDNA Kits
- QIAamp Circulating Nucleic Acid Kits

NGS/ Digital PCR

Next-generation sequencing library preparation solutions or digital PCR analysis solutions for various applications: Mutation analysis (screening and low variant somatic or hereditary mutation analysis by targeted NGS or digital PCR), whole genome sequencing, CNV and methylation analysis.

NGS

- QIAseq® Targeted DNA Panels
- QIAseq Targeted Methyl Panels
- QIAseq Human Exome Kits
- QIAseq cfDNA Library Kits

Digital PCR

- QIAcuity® dPCR Systems
- dPCR LNA Mutation Assays
- dPCR Copy Number Assays

Data analysis

Complete NGS and ‘omics data analysis tools with flexible access. Rapid, evidence-powered variant annotation for human exome, genome, and large cohort sequencing data.

- QIAGEN® CLC Genomics Workbench

Interpretation

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

- COSMIC
- QCI® Interpret Translational for QIAseq
- QIAGEN Ingenuity Pathway Analysis

Genomic Services

- Extend your in-house resources quickly and conveniently
- Ensure your data is high quality using our expertise and tailored services
- With our unique spectrum of innovative technologies, get support with just the time-consuming parts of your project or with your entire Sample to Insight journey
- Benefit from expert consultation and make the best decisions to achieve your goals

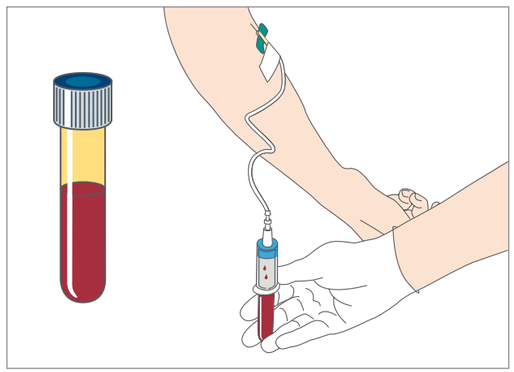
GeneGlobe® Design & Analysis Hub

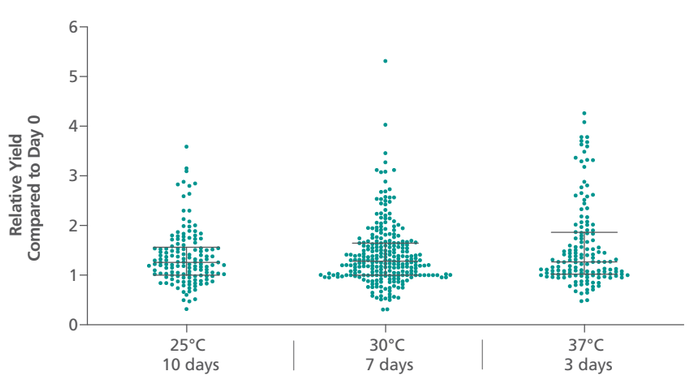
- Quickly and easily explore targets in their scientific context
- Find and customize the right products to study those targets
- Analyze the data and plan follow-up studies

Sample collection, stabilization and cfDNA isolation for any throughput

PAXgene Blood ccfDNA Tubes

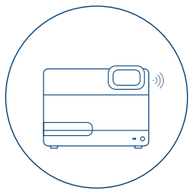
- Single tube collection, stabilization, transport and storage
- Formaldehyde-free, non-crosslinking stabilization of circulating, cell-free DNA (cfDNA) in plasma and genomic DNA in the nuclear cellular fraction
- Length of stabilization:
  - 2–37°C for up to 3 days
  - 2–30°C for up to 7 days
  - 2–25°C for up to 10 days





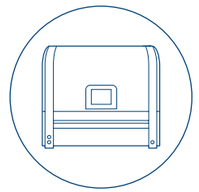
Minimization of DNA release into plasma from blood samples stored in the PAXgene Blood ccfDNA Tubes. Change in cfDNA yield after blood storage in comparison to plasma separated within 2 hours of blood collection (Day 0). Blood was stored for 10 days at 25°C (n = 141 subjects), 7 days at 30°C (n = 261) or 3 days at 37°C (n = 144) followed by centrifugation and ccfDNA purification using the automated QIAsymphony PAXgene Blood cfDNA Kit or the manual QIAamp DSP Circulating Nucleic Acid Kit. The relative cfDNA yield of 18S rDNA copy numbers is shown after sample storage compared to copy numbers at Day 0.

EZ2 Connect



- Purify cfDNA from 1–24 samples fully 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 cartridges

QIAsymphony SP



- Fully automated cfDNA sample preparation of 1–96 samples
- Continuous sample loading, with bar code reading for sample tracking
- Ready-to-run reagent cartridges prefilled with all reagents required for the purification
- Validated protocols for 2 ml and 4 ml, customized protocols for large volumes (up to 10 ml) for molecular biology applications.
- Import of sample lists and export of sample sheets
- Primary tube handling option allows direct processing of primary blood collection tube

QIAcube Connect

- Automates over 80 QIAGEN kits with over 140 standard protocols for DNA, RNA and protein sample processing
- Up to 10 ml input volume
- High concentrations due to elution volumes down to 20 µl
- QIAsphere ready: monitor runs remotely, for quick response times, through the QIAsphere App

Sample preparation solutions for research applications

	Starting volume	Processing time	Throughput (samples per run)	Instrument	Eluate (µl)	Comments
QIAamp MinElute ccfDNA Mini Kit (50), cat. no. 55204	1–4 ml	45 min		Manual	20	Highly concentrated cfDNA from plasma or serum due to low elution volume
QIAamp MinElute ccfDNA Midi Kit (50), cat. no. 55284	5–10 ml	70 min	Up to 12	QIAcube Connect	30	Low frequency variant detection enabled with scalable sample input volumes (up to 10 ml). Elution volume can be reduced to 20 µl.
QIAamp Circulating Nucleic Acid Kit (50), cat. no. 55114	Up to 5 ml Up to 4 ml	<2 h		Manual using vacuum with QIAvac 24 Plus	20–150	Purification of free-circulating DNA and RNA from plasma, serum or urine.
EZ1&2 ccfDNA Kit (48), cat. no. 954854	1–2 ml	35 min	Up to 24	EZ2 Connect	70	Isolation of cfDNA from serum and plasma samples of up to 8 ml starting volume.
	3–4 ml	46 min				Fully automated cfDNA preparation on EZ2 Connect (and EZ1 Advanced XL instrument).
	5–8 ml	70 min				Highly flexible cfDNA sample preparation of 1 to 24 samples in parallel in just 35–70 minutes.
QIAsymphony PAXgene Blood ccfDNA Kit (192)*†, cat. no. 768536	2.4 ml	1 h 5 min	24	QIAsymphony SP	60, 100, or 150	Optimized isolation chemistry for plasma generated in PAXgene Blood ccfDNA Tubes.
		4 h 20 min	96			Primary tube handling protocols for 2.4 and 4.0 ml plasma allow direct placement of the centrifuged PAXgene Blood ccfDNA Tubes onto the QIAsymphony SP.
	4.0 ml OR 4.8 ml	1 h 25 min	24			Separate protocol lines for small (STA) and large (LAF) cfDNA fragments.
		5 h 40 min	96			

* Not available in all countries.

† Customized protocols for large volumes available (only for molecular biology applications).

plasma/serum urine

Sample preparation solutions for diagnostic applications

	Starting volume	Processing time	Throughput	Instrument	Eluate (µl)	Comments
QIAamp DSP Circulating NA Kit (50)*, cat. no. 61504	Up to 5 ml	<2 h		Manual using vacuum with QIAvac 24 Plus	20–150	Purification of cell-free DNA and RNA from plasma for in vitro diagnostic use.
QIAsymphony DSP Circulating DNA Kit (192)*†, cat. no. 937556	2 ml OR	70 min	24	QIAsymphony SP	60	Automated purification of cfDNA from human plasma and urine.
		4 h 40 min	96			
	4 ml OR	90 min	24		60	Workflow compatibility with a wide range of cfDNA stabilizing and non-stabilizing blood collection Tubes (cat. no. 76815).
		6 h	96			
QIAsymphony PAXgene Blood ccfDNA Kit (192)*‡, cat. no. 768566	2.4 ml	1 h 5 min	24		60	Primary tube handling protocols for 2.4 and 4.0 ml plasma allow direct placement of the centrifuged PAXgene Blood ccfDNA Tubes onto the QIAsymphony SP.
		4 h 20 min	96			
	4.0 ml OR 4.8 ml	1 h 25 min	24		60	
		5 h 40 min	96			

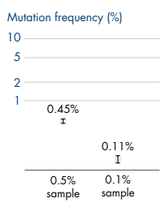
plasma/serum urine

* Not available in all countries.

† Customized protocols available on demand for higher starting volumes and for lower and higher elution volumes (only for molecular biology applications).

‡ Intended for automated isolation and purification of circulating cell-free DNA (ccfDNA) from plasma generated from human venous whole blood collected in the PAXgene Blood cfDNA Tube.

Cell-free DNA analysis by digital PCR

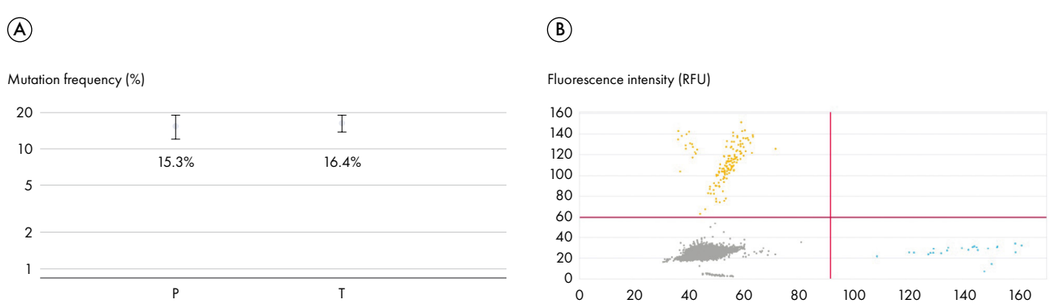


Highly sensitive detection at 0.1% mutation frequency. To test the sensitivity of the dPCR LNA Mutation Assay for PIK3CA p.C420R, an artificial sample was used. The two samples were set up in a (mutant/WT) ratio of 0.5% and 0.1% mutant fragment and run using QIAcuity Nanoplate 26k 24-well.

Variant detection with highest sensitivity

- Duplex assay design detects mutated and wild-type sequences
- Choice of FAM and HEX or Atto 550 and ROX fluorescent dye combinations
- LNA-enhanced primers and probes increase assay specificity and sensitivity
- Assays are dPCR wet-lab tested down to 0.1% VAF

dPCR LNA Mutation Assays, cat. no. 250200



Precise mutation analysis from plasma samples. PIK3CA p.C420R mutation detection using dPCR LNA Mutation Assays on QIAcuity. For the dPCR run on the QIAcuity, 2 µl DNA sample extracted from breast cancer patient samples (FPPE tissue as well as plasma sample) were loaded into two single wells of Nanoplate 26K 24-well. Genomic DNA was extracted from two FFPE sections using the QIAamp DSP DNA FFPE Tissue Kit (elution volume 20 µl) and ctDNA from 2 ml plasma using the QIAamp DSP Circulating Nucleic Acid Kit. **A**: Mutation frequencies showing strong concordance between plasma (P) and FFPE tissue (T) samples. **B**: 2D scatter plot of a single well from the Nanoplate 26k of one plasma sample. The plot shows clear separation of positives and negatives. Gray cluster: negative partitions; blue cluster: positive partitions for the mutated DNA only, yellow cluster: positive partitions for wild-type DNA only.

Copy number change analysis for an individual gene or region of interest

- Predesigned assays for all genes in the human genome
- Choice of 3 design locations per gene: 5', middle and 3'
- Convenient copy number data analysis using the QIAcuity Software Suite

dPCR Copy Number Assays, cat. no. 250205

Nanoplate-based digital PCR

- Microfluidic, nanoplate based, fully integrated digital PCR systems
- Scalable format (1-, 4- and 8-plate instruments) with advanced multiplexing capabilities (up to 5plex)
- Comprehensive results in under two hours with workflow and hands on as in qPCR
- Nanoplates with 26K or 8.5K partitions

QIAcuity dPCR systems, cat. no. varies

Impactful discoveries powered by NGS

DNA sequencing of your region of interest

- A wide range of catalogued, extended, booster or custom DNA panels
- Low frequency variant detection with unique molecular indices (UMIs) and single primer extension (SPE)
- Unique Dual Indices to reduce index hopping and read misassignment

QIAseq Targeted DNA Pro Panels, cat. no. 333651

Targeted DNA methylation analysis by NGS

- Liquid biopsy-compatible solution for targeting methylation status of DNA from as low as 10 ng cfDNA input
- PCR error correction with UMIs
- SPE approach that increases sensitivity

QIAseq Targeted Methyl Panels, cat. no. 335501

Whole genome sequencing of cfDNA

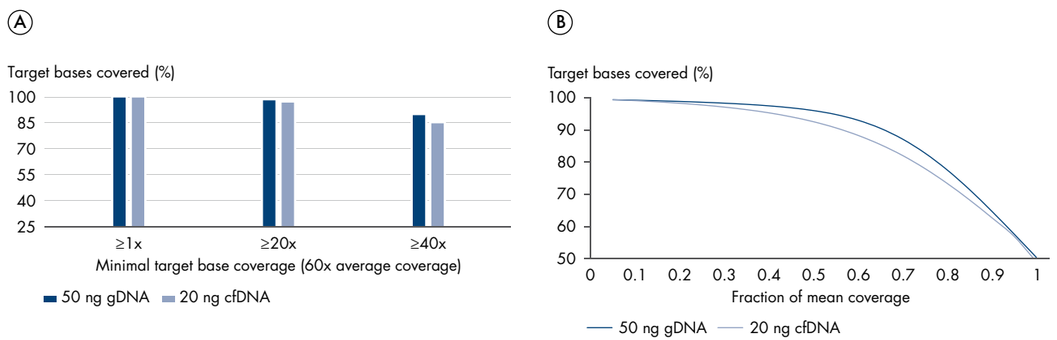
- Specially optimized for cfDNA whole genome sequencing
- Go directly from eluant to library prep without quantification using 1–100 ng range of cfDNA input
- Minimize PCR with high-fidelity amplification reagents or generate PCR-free libraries from just 10 ng of cfDNA

QIAseq cfDNA Library Kits, cat. no. 180015

Exome library preparation

- Single-day, automation-compatible sample to sequencing workflow
- Delivering exceptional coverage uniformity with variable hybridization times (even from FFPE and cfDNA samples)

QIAseq Human Exome Kits, cat. no. 333937



Analysis of whole exome libraries from cfDNA samples. Plasma-derived free circulating DNA was isolated using the QIAamp MinElute ccfDNA Mini Kit. After whole genome library preparation from 20 ng cfDNA using the QIAseq cfDNA Library Kit, the QIAseq Human Exome Kit was used for whole exome enrichment of the samples. **A:** Minimal base coverage. **B:** Uniformity plot. Despite the reduced input amount and the challenging nature of cfDNA, the resulting data were of adequate quality and comparable to data obtained from gDNA.

Expert curated genomic and clinical knowledge, together with bioinformatics software and services, for actionable insights

QIAGEN CLC Genomics

Complete NGS and 'omics data analysis tools with flexible access, from a laptop to a local server to the cloud. Powerful analysis easy to learn for non-bioinformaticians.

QIAGEN Ingenuity Pathway Analysis (IPA)

Quickly visualize and understand complex 'omics data and perform insightful data analysis and interpretation by placing your experimental results within the context of biological systems.

COSMIC

World's largest source of expert manually curated somatic mutation information relating to human cancers the database attempts to collate all known (published) genetic mutations responsible for human cancers.

QCI Interpret Translational

Rapid, evidence-powered NGS variant annotation, filtering and triage.

Learn how to integrate your cfDNA workflows: **qiagen.com/cfDNA-workflow**

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