PAXgene® Blood ccfDNA

PAXgene Blood ccfDNA Tube (RUO)
QIAsymphony PAXgene Blood ccfDNA Kit (RUO)

For Research Use Only. Not for use in diagnostic procedures.

Explore more at **www.preanalytix.com**

PreAnalytiX
A QIAGEN / BD Company

Situation

Working with circulating cell-free DNA (ccfDNA) presents specific challenges in preserving levels of ccfDNA for accurate analysis in research applications including the evaluation of the fetal DNA fraction in maternal blood and the examination of circulating tumor DNA. Because ccfDNA is present in low quantities in blood, care must be taken to ensure that appropriate collection and isolation methods are used to avoid further dilution by genomic DNA from apoptotic or lysed cells. Without proper blood cell stabilization, the level of cell-free DNA can change dramatically if plasma is not separated immediately after blood collection and stored under proper conditions.

Solution

Minimize negative downstream impacts caused by blood collection tubes
In addition to the release of genomic DNA from dying white blood cells, different anticoagulants (EDTA, citrate or heparin) and crosslinking reagents in blood collection tubes can cause variation in the quality and integrity of ccfDNA isolated from blood specimens. PAXgene Blood ccfDNA Tubes contain a reagent that stabilizes ccfDNA levels in plasma without impacting downstream assays.

Reduce variability due to different shipping and storage conditions
PAXgene Blood ccfDNA Tubes contain an additive that anticoagulates blood and stabilizes blood cells via a non-crosslinking stabilization solution. The additive helps inhibit cell apoptosis, minimizes the release of intracellular DNA and stabilizes ccfDNA levels in plasma.

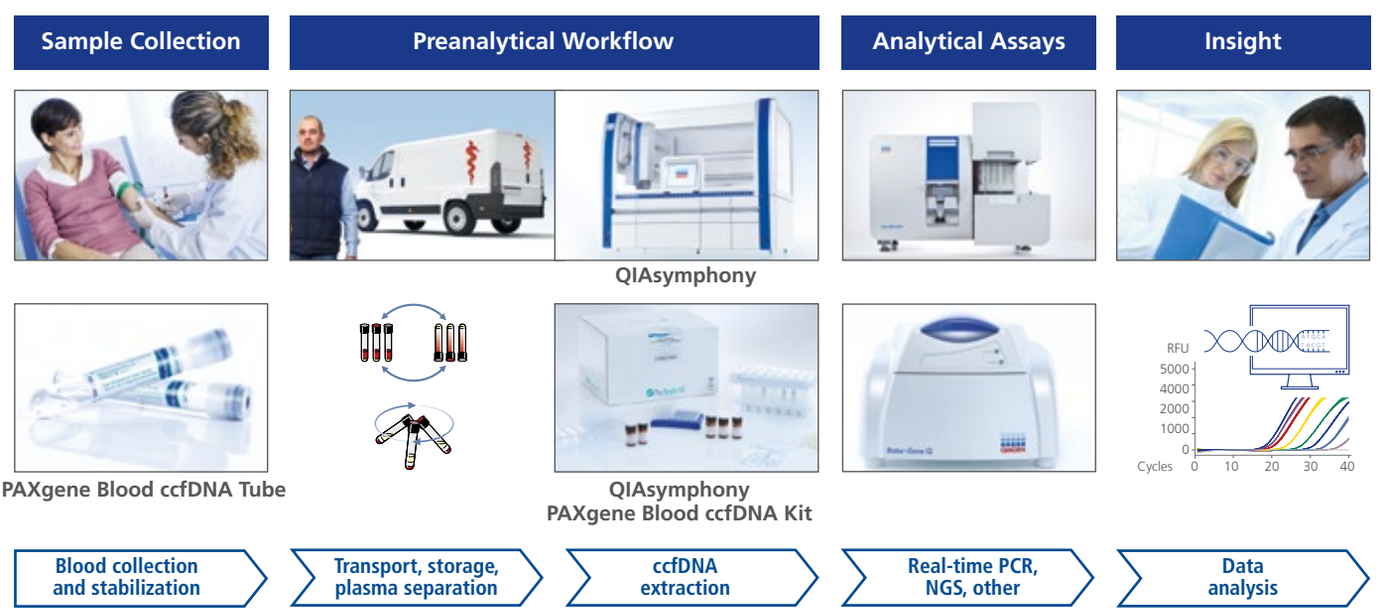
Straightforward coupling with ccfDNA purification
Blood collected into PAXgene Blood ccfDNA Tubes can be used for isolation of ccfDNA from plasma on the QIAsymphony® SP instrument. The fully automated QIAsymphony PAXgene Blood ccfDNA Kit delivers optimized system performance and offers the option of primary tube handling. Alternatively, ccfDNA isolation can be done manually using QIAGEN® QIAamp® technology, or semi-automated using the QIAGEN EZ1® instrument and kits.

PAXgene Blood ccfDNA System (RUO)

The PAXgene Blood ccfDNA System (for Research Use Only) consists of a blood collection tube (PAXgene Blood ccfDNA Tube) and a ccfDNA purification kit for the QIAsymphony SP instrument (QIAsymphony PAXgene Blood ccfDNA Kit).

For collection, storage and transport of blood, the stabilization of ccfDNA in a closed tube and the subsequent isolation and purification of ccfDNA from plasma for research applications.

PAXgene Blood ccfDNA System Workflow



Stabilization of ccfDNA profiles. The PAXgene Blood ccfDNA Tube features a proprietary stabilization reagent that targets and stabilizes blood cells so analyses reflect ccfDNA levels at the time of collection.

Standardized collection and preanalytical processing. The PAXgene Blood ccfDNA Tube uses BD Vacutainer® technology to consistently collect high-quality blood specimens while helping ensure healthcare worker and patient safety. Straightforward integration with companion manual or automated ccfDNA isolation streamlines the preanalytical workflow and delivers consistent results.

PAXgene Blood ccfDNA Tube (RUO)

Simplify and standardize the collection and processing of whole blood for subsequent purification of circulating, cell-free DNA (ccfDNA) for research purposes.

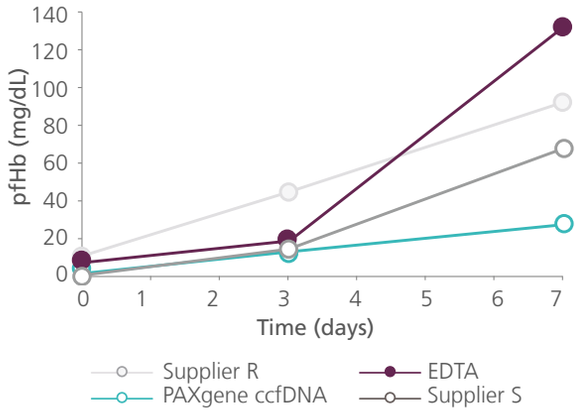
- Single tube collection, stabilization, transport and storage
- Immediate stabilization of circulating, cell-free DNA (ccfDNA) profile
- Stabilization of ccfDNA for days at various temperatures facilitates transport and storage
- Standardized preanalytical processing of samples

Specifications	
Tube size	16 × 100 mm
Blood draw volume	10 ml
Additive volume	1.5 ml
Sample stability	For up to 10 days at temperatures up to 25°C, 7 days at temperatures up to 30°C, or 3 days at temperatures up to 37°C prior to processing for plasma separation. Note: Do not store blood-filled tubes below 2°C
Closure type/color	Pearl white BD Hemogard™ closure with blue stopper
Quantity	100 tubes/case
Tube shelf life	15 months from date of manufacture

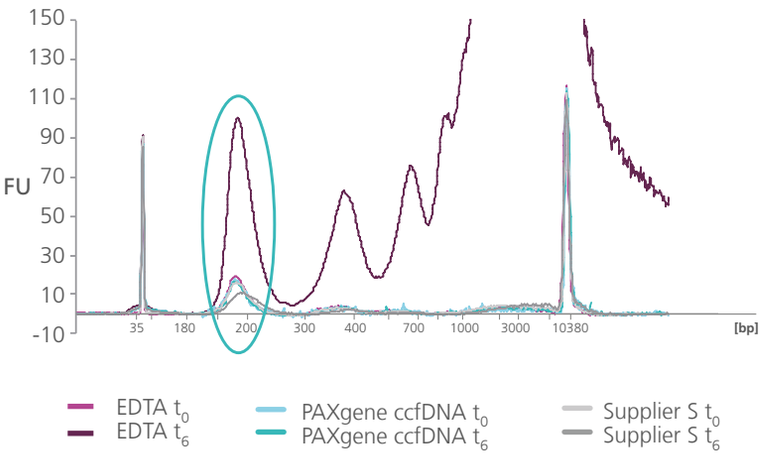
The PAXgene Blood ccfDNA Tube, in conjunction with the **QIAsymphony PAXgene Blood ccfDNA Kit** or QIAGEN **QIAamp** or **EZ1 ccfDNA** technologies, enables isolation of ccfDNA suitable for demanding downstream analytical research assays.

Feature	Benefit
Unique stabilization reagent	✓ Non-crosslinking preservation of extracellular levels of ccfDNA ✓ Distinct plasma separation due to minimal hemolysis of red blood cells
BD Vacutainer plastic tube and BD Hemogard safety closure	✓ Minimized risk of tube breakage ✓ Enhanced healthcare and laboratory personnel safety
Integrated preanalytical workflow	✓ Seamless integration with QIAsymphony ccfDNA, QIAamp or EZ1 ccfDNA technology ✓ Wide spectrum of research applications, including analysis of cancer tumor DNA and non-invasive prenatal testing ✓ No pretreatments, extra incubation times or added protocol steps
Primary tube handling option on the QIAsymphony SP instrument	✓ Reduced consumables, costs and biohazardous waste because secondary tubes are eliminated ✓ Shortened workflow – only 20 minutes for the preparation of 24 tubes ✓ Reduced risk of exposure and sample mislabeling

Effective stabilization of white and red blood cells minimizes gDNA release and reduces hemolysis during sample storage

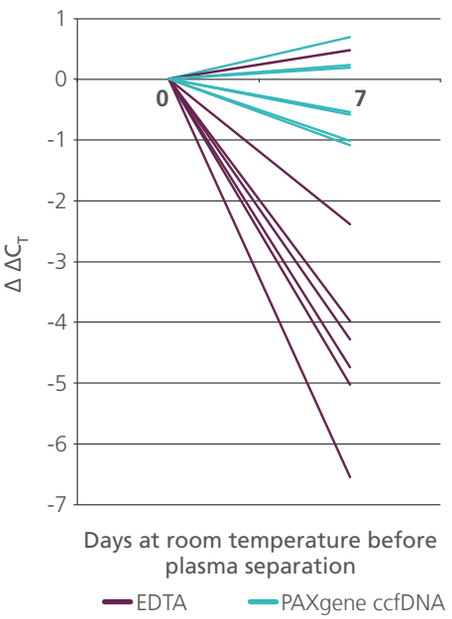


PAXgene Blood ccfDNA stabilization minimizes hemolysis compared to alternative solutions.
Whole blood from 20 subjects was collected into PAXgene Blood ccfDNA Tubes, EDTA tubes and blood collection tubes designed for ccfDNA stabilization from 2 other suppliers. Plasma was separated directly after blood draw and after storage at room temperature (15–25°C). Increase in sample hemolysis during sample storage at room temperature was minimized in PAXgene Blood ccfDNA Tubes compared to the other blood collection tubes.

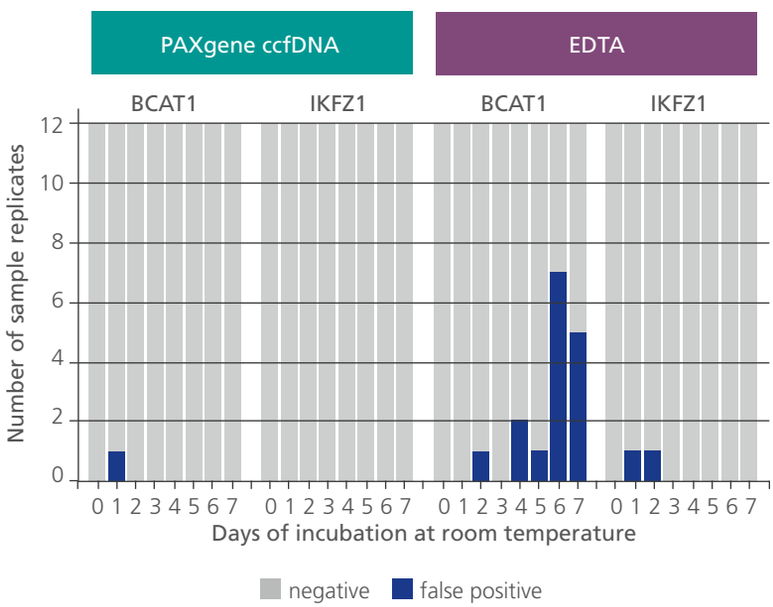


PAXgene Blood ccfDNA stabilization reagent helps prevent release of gDNA into plasma and leaves ccfDNA chemically unmodified.
Whole blood was stored in EDTA tubes, PAXgene Blood ccfDNA Tubes or tubes from Supplier S. ccfDNA was extracted from the plasma immediately following blood collection (t_0) or after 6 days storage (t_6) at room temperature. Plasma from EDTA tubes showed an increase in apoptotic gDNA fragments. Plasma from the Supplier S tubes shows a broadening and shift of the main ccfDNA peak towards larger fragments, indicating DNA modifications. Plasma from PAXgene Blood ccfDNA Tubes showed a ccfDNA profile comparable to day 0.

The non-crosslinking reagent is compatible with a wide spectrum of downstream applications including DNA methylation assays



Accurate detection of methylated circulating tumor (ct)DNA markers after storage.
Blood from lung cancer patients under treatment was collected into EDTA and PAXgene Blood ccfDNA Tubes. Plasma was separated either directly after collection or after storing whole blood for 7 days at room temperature. ccfDNA was then isolated from the plasma. PCR results from 7 donors with valid mSHOX2 signals (C_T<37) were analyzed. A ΔC_T was calculated from the mSHOX2 and the ACTB C_T values (C_T mSHOX2 – C_T ACTB). A $\Delta\Delta C_T$ was built with these ΔC_T values (ΔC_T t_0 – ΔC_T t_7) to visualize the relative signal change of mSHOX2 over time. Signal changes were significantly greater in blood samples stored in EDTA compared to samples stored in PAXgene Blood ccfDNA Tubes (data courtesy of Dr. Fleischhacker, UKH Halle/Saale).



Minimal false positive callings over storage time.
Whole blood of 4 healthy donors was collected into either EDTA or PAXgene Blood ccfDNA Tubes. Blood was left at room temperature for up to 7 days. Each day, ccfDNA was isolated from samples, bisulfite converted (Epitect FAST kit, QIAGEN) and analyzed with the GEMINI assay (Clinical Genomics) for 2 methylated targets (BCAT1 and IKZF1) and 1 control target (ACTB). Three PCR replicates per target sequence were analyzed. ccfDNA from blood stored in EDTA tubes generated more false positives than when blood was stored in PAXgene Blood ccfDNA Tubes (data courtesy of Clinical Genomics Technologies Pty Ltd.).

QIAsymphony PAXgene Blood ccfDNA Kit (RUO)

Discover the benefits of an integrated system approach combined with the ease and consistency of automation.

The fully automated QIAsymphony PAXgene Blood ccfDNA Kit features chemistry designed specifically to maximize recovery of ccfDNA from plasma processed from whole blood stabilized in PAXgene Blood ccfDNA Tubes.

- Optimized binding chemistry and input volumes for PAXgene Blood ccfDNA Tubes
- Separate protocol lines for small (STA) and large (LAF) ccfDNA fragments
- Prefilled reagent cartridges with bar code for safety and ease of use
- Standardized processing for high reproducibility
- Onboard measures of the QIAsymphony SP minimize cross-contamination

Specifications	
Format	Magnetic beads
Technology	Magnetic particle technology
Sample input volume	2.4 ml or 4.8 ml plasma*
Elution volume	60 µl for the STA protocol line* 60, 100 or 150 µl for the LAF protocol line*
Time per run	96 samples with 4.8 ml plasma in 5 h 40 min
Throughput	192 samples per working day and instrument
Processing	Manual: centrifugation Automated: QIAGEN QIAsymphony SP Instrument
Shelf life of open reagent cartridge	4 weeks

* Custom protocols for other input and elution volumes are available upon request.

During the QIAsymphony SP run, plasma proteins are digested by proteinase K while ccfDNA binds to the surface of magnetic beads. Depending on protocol line, predominantly small ccfDNA fragments (STA protocol line) or small and large ccfDNA fragments (LAF protocol line) are isolated. Three wash steps ensure contaminant removal. Finally, ccfDNA is eluted from the magnetic particles and is ready for use in downstream applications.

The QIAsymphony PAXgene ccfDNA Kit generates yields comparable to the QIAamp Circulating Nucleic Acid Kit, providing consistent and comparable results.

Study A	
Comparison to manual QIAamp Circulating Nucleic Acid Kit	
Study parameters	
Samples tested	825
Donors	189
Runs	42
Instruments	6
Study results	
ccfDNA yield*	104%
Portion of dsDNA†	>96%
Study B	
Evaluation of system performance	
Study parameters	
Samples Tested	1636
Donors	204
Runs	71
Instruments	6
Study results	
Yield compared to EDTA plasma‡	No significant difference
ccfDNA stability at room temperature ¶	7 days
Cross Contamination	No evidence
Eluate freeze/thaw cycles	3
Sample throughput per day§	192
Open reagent cartridge shelf life	4 weeks
Runtime for 96 samples	5 h 40 min

* Because yield is strongly dependent of donor, individual yields may vary.

† Tested with a restriction enzyme-based assay for a subset of eluates; QIAamp Circulating Nucleic Acid Kit eluates with comparable portion of double-stranded DNA (dsDNA).

‡ Tested directly after blood collection with 60 samples from 20 donors.

¶ Tested with 60 samples from 20 donors.

§ Includes plasma processing and overnight runs.

Ordering Information

Product	Contents	Cat. no.
PAXgene Blood ccfDNA Tube (100) (RUO)*	100 blood collection tubes (10 ml). To be used in conjunction with QIAamp Circulating Nucleic Acid Kit or QIAsymphony PAXgene Blood ccfDNA Kit	768115
QIAsymphony PAXgene Blood ccfDNA Kit (192) (RUO)*	Reagent cartridges, accessories and proteinase K vials for 192 preps	768536
QIAamp Circulating Nucleic Acid Kit†	For 50 preps: QIAamp Mini Columns, tube extenders (20 ml), QIAGEN proteinase K, carrier RNA, buffers, VacConnectors and collection tubes	55114
QIAamp MinElute ccfDNA Kits (50)†	For 50 preps: QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, buffers, bead elution tubes, collection tubes	55204 Mini 55284 Midi
EZ1 ccfDNA Kits (48)†	For 48 preps: reagent cartridges, QIAGEN Proteinase K, Magnetic Bead Suspension, buffers, bead elution tubes, disposable tips	954134 Mini 954154 Midi
Accessories		
BD Vacutainer UltraTouch™ Push Button Blood Collection Set	23G ¾ inch (0.6 × 19 mm) needle, 12 inch (305 mm) tubing with luer adapter. 50/box, 200/case	367364
BD Vacutainer Safety-Lok™ Blood Collection Set	21G ¾ inch (0.8 × 19 mm) needle, 12 inch (305 mm) tubing with luer adapter. 50/box, 200/case	367281 US 367286 CE
BD Vacutainer One Use Holder	Case only for 13 mm and 16 mm Diameter, 1000/case	364815

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† Intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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Discover more about the PAXgene Blood ccfDNA products at: **www.preanalytix.com**

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