stratec molecular

From Liquid Biopsy and FFPE Samples to Results

Fully automated purification, reliable quantification and bisulfite conversion of cell-free circulating DNA and DNA from FFPE samples

Fully automated purification, reliable quantification and bisulfite conversion of cell-free circulating DNA or of DNA from FFPE samples

Liquid biopsy is a new technology for detection and analysis of biomarkers in blood or other body fluids without the need of invasive procedures. One important analyte is cell-free circulating DNA (cfDNA). The main challenges in working with cfDNA are the low concentration (1 - 50 ng/ml) and the high degree of fragmentation of cfDNA (< 500 bp). An additional complication is that the circulating tumor DNA only accounts for up to 20 % of the total cfDNA. Therefore processing higher sample volumes, typically in the range of several milliliters, is required. In order to address these obstacles STRATEC Molecular has developed a suite of products to

- Extract low amounts of fragmented DNA from 4 ml of plasma samples or from FFPE slides, fully automated on the InviGenius® PLUS instrument
- Reliably quantitate the extracted DNA using qPCR
- Perform fully automated bisulfite conversion of DNA on the InviGenius® PLUS instrument

These innovative solutions enable a reliable and reproducible application of cfDNA especially in the areas of oncology, extraction and analysis of fetal DNA from maternal plasma or improving the monitoring of transplant rejections. The individual products are designed to be combined in one efficient and robust workflow or as stand-alone process steps.

1. Automated extraction of circulating cell-free DNA

The **InviMag® Free Circulating DNA Kit/ IG** enables efficient, fully automated purification of free circulating DNA fragments from 4 ml of plasma or urine samples on the **InviGenius® PLUS**. The walk-away robotic system simplifies laboratory workflows and enables fully automated, standardized and highly efficient purification procedures.

BENEFITS

- Walk-away, highly reproducible and standardized cfDNA extraction from high sample volume (concentration from 4 ml down to 100 µl)
- Efficient recovery of short and fragmented cfDNA
- Only 10 minutes of hands-on time compared to at least 60 minutes for manual methods

PRODUCT SPECIFICATIONS

Protocol:	Magnetic bead based isolation of cfDNA on the **InviGenius® PLUS** platform
Starting material:	4 ml of plasma, urine or plasma from stabilized blood samples, e.g. Cell-Free DNA BCT® tubes from Streck
Throughput:	1 - 12 samples per run
Yield of cfDNA:	15 - 150 ng from 4 ml plasma
Processing time:	2.5 - 3.5 h

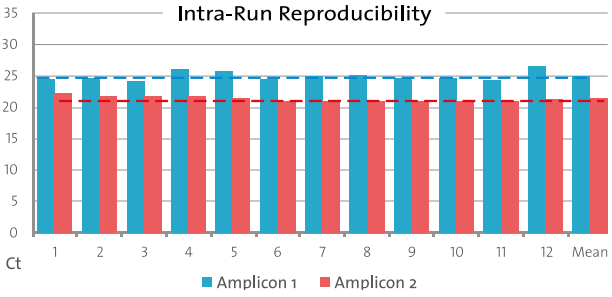


Fig. 1: Excellent and reproducible intra-run recovery of circulating cfDNA

*Circulating cell-free DNA was isolated from 12 aliquotes of 4 ml Seracon plasma samples in parallel using the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS** and eluted in 100 µl. DNA yield was quantified by real-time PCR of two amplicons within the 18S rRNA sequence.*

FAM (70 bp) CT Std. Dev: 0.72 Cy5 (177 bp) CT Std. Dev: 0.33

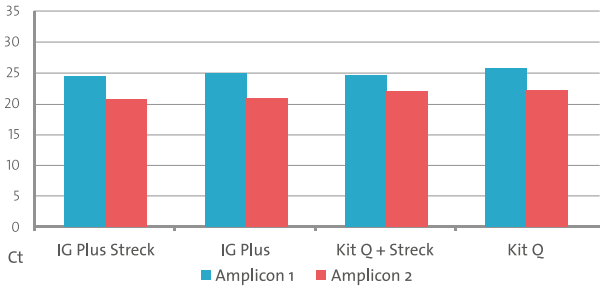
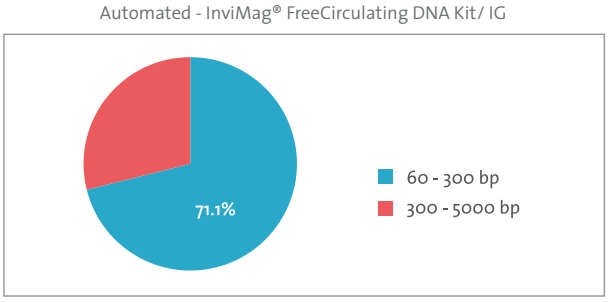


Fig. 2: Comparison of extraction efficiency from stabilized samples using automated and manual kits

*cfDNA was isolated from 4 ml plasma (same blood collected in Streck tubes and EDTA tubes) using the fully automated procedure of the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS** in comparison to a manual kit. DNA was quantified by real-time PCR of two amplicons within the 18S rRNA sequence (70 bp & 177 bp). Samples collected with Streck tubes yield comparable results to conventional EDTA tubes and the extraction efficiency is at least comparable to competition.*



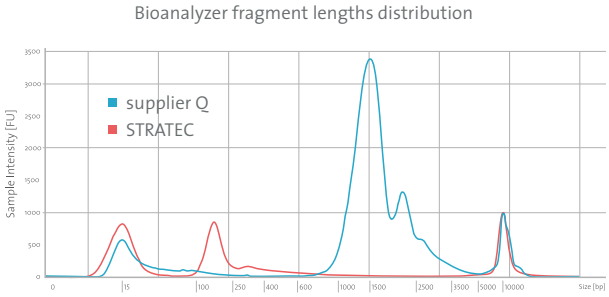


Fig. 3 a+b: cfDNA fragment lengths distribution using automated & manual extraction methods

*3a. cfDNA was isolated from 40 different plasma samples of patients diagnosed with cancer of different origin and stages using the **InviMag® Free Circulating DNA Kit/ IG** on the **InviGenius® PLUS.***
*The automated kit on the **InviGenius® PLUS** yields a proportion of over 70% small cfDNA fragments from 60 – 300 bp which are of higher interest for subsequent biomarker analysis in oncology.*

*3b. Example of cfDNA fragment lengths analysis using the Bioanalyzer TapeStation HSD 5000. Comparison of cfDNA extracted from identical sample using the **InviMag® Free Circulating DNA Kit/ IG** on **the InviGenius® PLUS** and the manual kit from supplier Q.*

2. Standardized & fully automated extraction of DNA from FFPE tissue

Genotyping tumor tissue in search for genetic alterations has become routine practice in research and clinical oncology. Since FFPE samples as a source for cancer testing became more widely adopted pathology laboratories are in need of automated solutions. The new **InviMag® FFPE DNA Kit/ IG*** enables efficient, fully automated purification of genomic DNA fragments direct from single paraffin embedded tissue samples (1-5 FFPE slides) on the **InviGenius® PLUS** without any manual pretreatment.

BENEFITS

- Fully automated extraction of DNA from FFPE samples - high reproducibility through standardized processing
- Efficient recovery even of short and fragmented ready-to-use DNA
- No manual pretreatment like deparaffination or formalin removal required
- Protocol can be integrated with automated bisulfite conversion
- Automated method saves 60 % of hands-on time

PRODUCT SPECIFICATIONS

Starting material:	1 - 5 FFPE slides
Protocol:	Magnetic bead based kit for DNA isolation, for use in combination with the InviGenius® PLUS
Throughput:	1 - 12 samples per run
Elution Volume:	80 µl input; 50 µl output
Yield:	2 - 20 ng (depending on sample)
Processing time:	3.5 - 4 h (including 2.5 h lysis time)

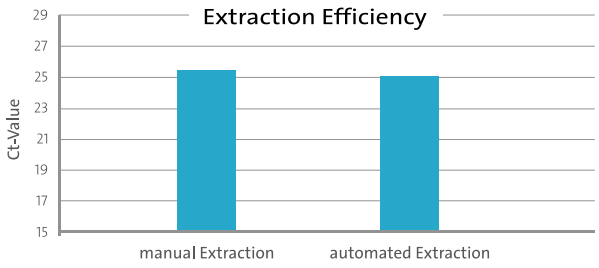


Fig. 4 Comparison of manual and automated FFPE extraction efficiency

*DNA was extracted from single FFPE slides of a primary tumor in triplets, manually using the **Invisorb® Spin Tissue Mini Kit** and fully automated using the **InviMag® FFPE DNA Kit /IG** (prototype) on the **InviGenius® PLUS** platform. The isolated DNA was used in a real-time PCR using the **InviQuant GeneCount 40** for DNA quantification. The graphic shows the average CT values (triplet) of the DNA yield from FFPE samples from the same tumor block. (The slides as well as the tissue amount in the slides vary slightly.)*

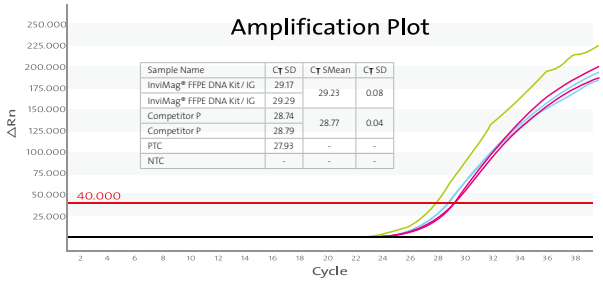


Fig. 5 Comparable results using different manual and automated isolation methods

*DNA was isolated from one FFPE slide of a primary tumor (duplet) using a manual kit from competitor P (blue curves) and fully automated using the **InviMag® FFPE DNA Kit /IG** on the **InviGenius® PLUS** (pink curves). The extracted DNA was analyzed in a K-ras specific real-time PCR. The amplification plot shows comparable CT values for both extraction methods (measured in duplicates).*

Pink: InviMag® FFPE DNA Kit /IG (prototype)
Blue: Competitor P
Green: Positive control
Black: Negative control

3. Quantification of DNA prior to cost intensive downstream applications

The **InviQuant GeneCount 40** is a qPCR-based system to determine the quantity of human genomic DNA e.g. prior to use in NGS or other downstream applications. Unlike other approaches that target a single genomic locus, the InviQuant GeneCount 40 uses a qPCR assay to detect 40 genomic loci which are randomly distributed throughout the human genome. The proprietary design ensures minimal variation caused by local genomic events. The use of the included high-quality DNA standard allows quantification of amplifiable targets in cfDNA samples.
Internal and external studies demonstrate that results for the quantification of cfDNA from plasma samples between the Qubit / Bioanalyzer and the quantitative PCR in many cases do not match. Differences of 50 to even 100% between the methods are fairly common. Since the qPCR assay directly measures the amplifiable portion of DNA it is the method of choice to analyze this specific sample type.

BENEFITS

- Provides qPCR based DNA quantification of up to 200 samples
- Quantifies isolated cfDNA or traces of genomic DNA that is amplifiable by PCR, suitable for determining the correct amount of input DNA for NGS application
- Increased sensitivity in comparison to single copy qPCR through detection of 40 randomly distributed genomic loci
- Method of choice for cfDNA quantification, qPCR assay directly measures the amplifiable portion of DNA

PRODUCT SPECIFICATIONS

Processing volume:	25 µl (5 µl DNA)
gDNA input concentration:	between 10 pg/µl to 2.5 µg/µl
Processing Time:	60 min (preparation and cycling)
Standard for quantification:	100.000 PCR product copies
Storage:	at – 20°C

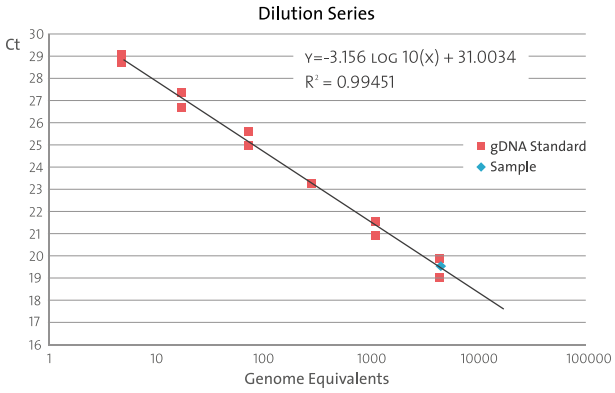


Fig. 6: Dilution series

*Genomic DNA with known quantities of genome equivalents (6 pg ≙ 1 diploid genome) was serially diluted. Serial dilutions were measured by qPCR using the **InviQuant GeneCount 40**. Ct-values were plotted against the known genome equivalents to generate a standard curve and its linear equation. All samples were measured in duplicates. Genome equivalents for the sample (blue) were calculated using the linear equation.*

E.g. 10^ (31.0034 – 19.5) / 3.156 = 4894 genome equivalents

4. Complete automated bisulfite conversion for methylation analysis

Due to the limited amount of DNA available, determination of methylation patterns in DNA from precious and limited sample materials e.g., formalin-fixed paraffin-embedded (FFPE) tissue or cfDNA is especially challenging. However, such sample types are of high interest for the successful identification of biomarkers. The **InviMag® Bisulfite Conversion Kit/ IG** features a fully automated and reliable bisulfite treatment and conversion of DNA and cfDNA for methylation analysis using the InviGenius® PLUS.

BENEFITS

- Fully automated processing on InviGenius® PLUS starting from DNA and cfDNA
- Standardized walk-away workflow starting from blood, FFPE or plasma sample to bisulfite conversion of the isolated DNA on the instrument possible
- Only 10 min hands-on time
- Conversion efficiency > 99.99 %

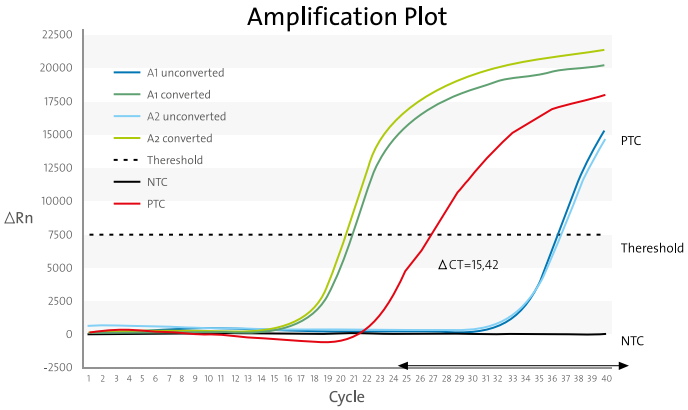


Fig. 7: Efficient cytosine conversion

*Two replicates of one sample (A1, A2) with each 1 µg of genomic DNA were converted using the **InviMag® Bisulfite Conversion Kit/ IG**. The amount of converted and unconverted DNA in each sample was determined by an in-house multiplex real-time PCR assay with conversion specific primers.*

CASE STUDY - Workflow from sample to result

cfDNA was isolated from 4 ml of six different liquid samples (plasma, serum or rinse liquid) with a predetermined status. The **InviMag® Free Circulating DNA Kit /IG** on the **InviGenius® PLUS** and a manual Spin kit from competitor Q were used for isolation. In parallel cfDNA was isolated from 200 µl of the same samples using a manual Spin kit from competitor R.

***Table 1:** The isolated cfDNA was quantified using the **InviQuant GeneCount 40** and results were converted to genome equivalents. The extraction methods using 4 ml samples volume provided comparable DNA yields.*

			InviMag® Free Circulating Kit /IG		Manual Kit Q		Manual Kit R	
Sample No.	Specimen	Status	Ct of cfDNA InviQuant GeneCount 40	Calc. Genome Equivalents	Ct of cfDNA InviQuant GeneCount 40	Calc. Genome Equivalents	Ct of cfDNA InviQuant GeneCount 40	Calc. Genome Equivalents
3	Rinse liquid	E.C.	28.61	6	29.82	2	33.58	0
10	Plasma	E.C.	26.65	25	25.86	45	28.73	5
18	Serum	O.C.	23.94	186	24.95	88	31.28	1
16	Serum	O.C.	22.18	680	23.01	369	26.52	28
2	Rinse liquid	O.C.	19.50	4894	20.35	2617	26.16	36
17	Serum	O.C.	17.14	27839	18.41	10924	25.72	50

Status: Ovarian Carcinoma = O.C: or Endometrium Carcinoma = E.C

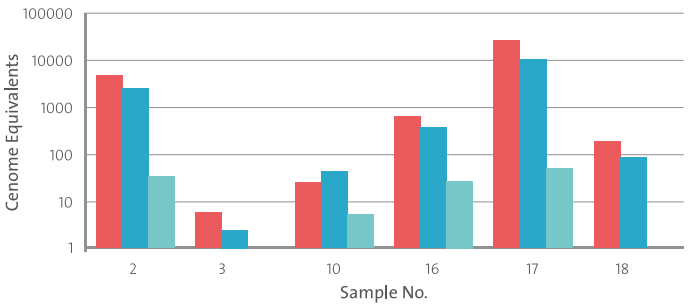


Fig. 8: Genome equivalents per sample and extraction system

- cfDNA Kit/ IG
- Competitor Q
- Competitor R

***Table 2:** Following quantification the cfDNA was bisulfite converted and analyzed for three specific methylation markers using qPCR. High sample volumes (4 ml; InviMag® Free Circulating DNA Kit /IG, competitor Q) generate more robust results and higher sensitivities compared to smaller sample volumes (200 µl; competitor R).*

	Sample 16	Sample 2			Sample 17
	Ct MM* 2	Ct MM* 1	Ct MM* 2	Ct MM* 3	Ct MM* 2
cfDNA Kit/ IG	32.68	23.85	24.58	23.49	33.21
cfDNA Kit, Q		23.49	24.33	23.74	
cfDNA Kit, R		27.75	29.16	27.15	35.28

MM = Methylation Marker
cfDNA Kit/ IG = InviMag® Free Circulating DNA Kit /IG

Data kindly provided by Dr. N. Häfner, Gynecological Molecular Biology, Department of Gynecology, Jena University Hospital, Germany

InviGenius® PLUS

The InviGenius® PLUS is a true walk-away system for DNA/RNA extraction and purification from clinical samples – providing a reliable "Sample in – Eluate out" technology! The combination of well-established magnetic bead based InviMag® technology and state-of-the-art process automation allows for standardization and streamlining of laboratory workflows. Innovative functionality and optimized protocols for demanding samples and applications result in reliable performance and superior DNA and RNA quality for molecular diagnostics*.

BENEFITS

- Extract and purify DNA and RNA from up to 12 liquid samples in parallel
- Direct processing from primary tubes
- Up to 4 ml sample volume
- Total in-process control
- Advanced process safety and standardized sample preparation
- CE-marked according to IVD-directive*

**) In compliance with the Directive 98/79/EC on in vitro diagnostic medical devices (IVD-Directive). Products which are CE-marked according to the IVD-Directive can be used for diagnostic applications in countries where this directive is recognized. The device is not approved by the US FDA.*

FEATURES

- Heat lysis and heat elution
- LIMS connectivity
- Choice of elution tubes or plates
- Barcoded labware for complete sample traceability
- Plug-in for separate hand-held barcode scanner
- Drop catcher minimizes the risk of cross contamination
- UV light enables decontamination of the worktable

Ordering information

Product	Package size	Catalogue number
InviMag® Free Circulating DNA Kit/ IG	8 x 12 purifications	2439320400
InviMag® FFPE DNA Kit/ IG	8 x 12 purifications	available Q3/2017
InviGenius® PLUS	1 piece	5011102000
InviMag® Bisulfite Conversion Kit/ IG	8 x 12 conversions	3030200100
InviQuant GeneCount 40	200 tests	3130100100

The kits are for research use only.

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617bq16/03/2017