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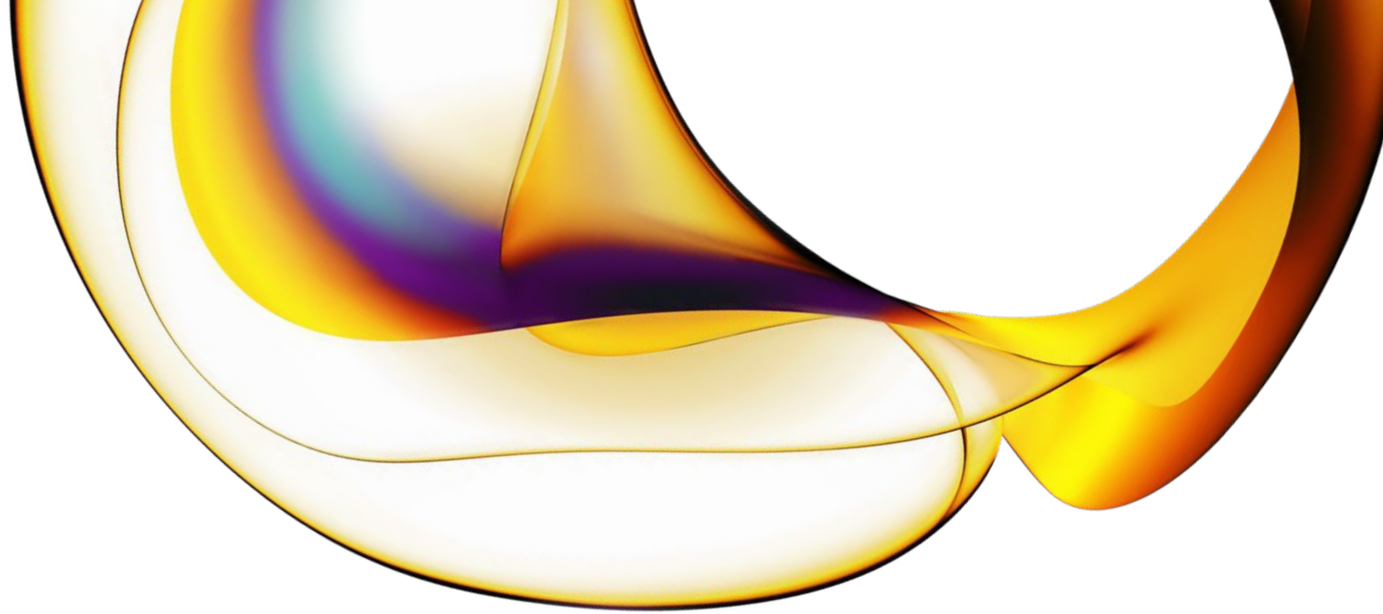
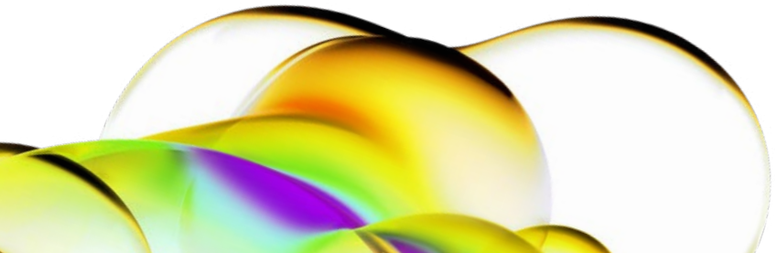
BIO-RAD



Coupling large volume chemagic™ cfDNA extraction with Droplet Digital™ PCR for high, consistent ctDNA detection.



Introduction

Analyzing circulating cell free DNA (cfDNA) from liquid biopsies can serve as a non-invasive biomarker, providing real-time insights into cancer disease state. The very low levels of highly fragmented cfDNA present in blood however, challenges detection. Assays that rely on detecting rare mutations in circulating tumor DNA (ctDNA) pose further limits on detection and is difficult to realize with conventional molecular techniques such as PCR. The routine implementation of cfDNA analysis as a reliable biomarker is additionally hampered by preanalytical variables that derive from sample collection, nucleic acid extraction, quantification, and quality control procedures.

Here, we describe a workflow that serves to counter these inherent challenges. To increase yields and improve consistency, the M-PVA Magnetic Bead based chemagic™ cfDNA extraction technology can be performed from large plasma volumes at scalable throughputs. For reliable quality control and to improve standardization across laboratories, the Mimix™ multiplex reference standard is applied with the option of post-extraction analysis on the LabChip® GX Touch for reliable sizing and cfDNA quantitation. Finally, for highly sensitive and precise detection of low abundance targets, Droplet Digital™ PCR (ddPCR™) is used that circumvents the need for calibration and removes PCR bias. These practical benefits are exemplified in our Field Application – Clinical Research Highlight – where we describe the use of chemagic cfDNA extraction with ddPCR at the Zealand University Hospital to support clinical research for lung, colorectal and anal cancer.

Workflow and Product Description

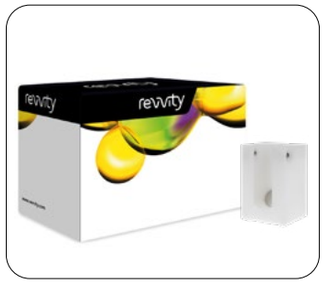
chemagic 360 instrument for automated cfDNA extraction

Automated or manual chemagic cfDNA extraction

The chemagic cfDNA extraction technology relies on proprietary M-PVA Magnetic Beads and obtains high yields of cfDNA comparable to column methods[1], while affording efficient scalability to large sample volumes or throughputs with chemagic automation.

- High, consistent cfDNA yields
- Increased likelihood of cfDNA detection through large sample volume processing (up to 18 ml) with chemagic automation
- Easily scale from manual to automated workflows with comparable results due to same kit chemistries
- Walk-away automation options available with full sample traceability and LIMS compatibilty

Visit our website to learn more.



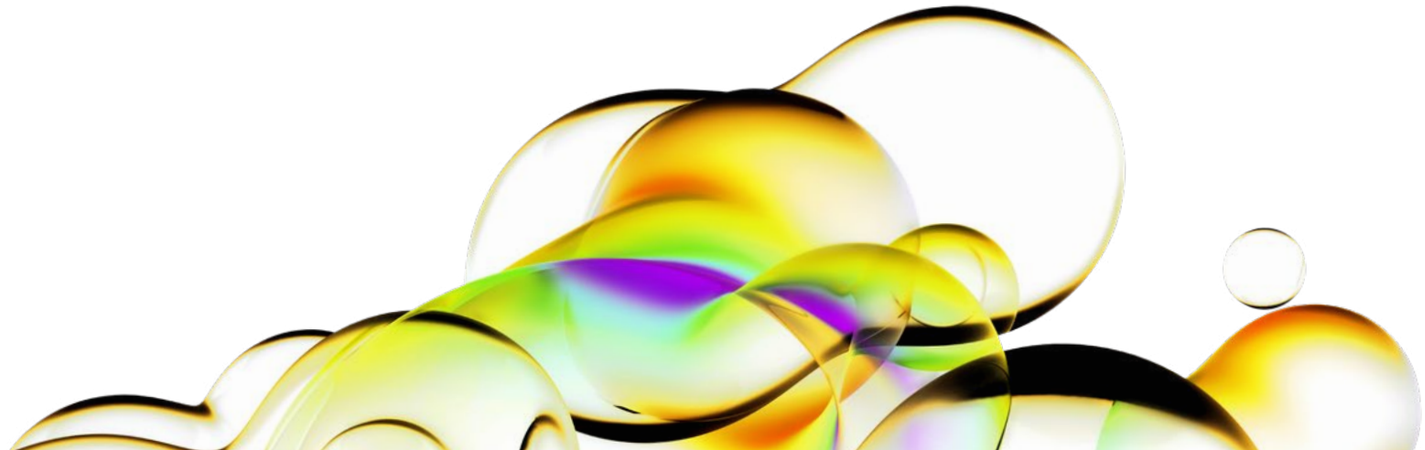
chemagic cfDNA 5K kit with magnetic Stand F for manual cfDNA extraction

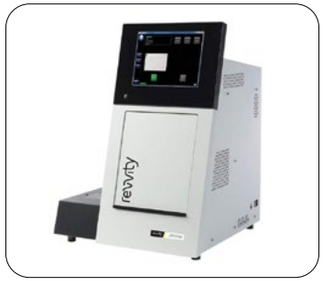
Mimix™ Multiplex I cfDNA Reference Standard in Synthetic Matrix II

This Mimix™ reference standard consists of fragmented DNA mimicking ctDNA, with genetically defined common cancer mutations spiked in synthetic plasma, providing reliable control of liquid biopsy assays.

- Contains 8 oncology relevant mutations to measure extraction efficiency and assay performance [2, 3].
- Biologically relevant cancer mutations in an artificial matrix give you full control of your cfDNA workflow, reducing the likelihood of a false negative or positive due to incorrect assay loading.
- Four different known range of allelic frequencies suitable for end-to-end process control.
- Compatible with commonly used extraction kits including bead and column-based methods, making it a highly flexible.

Visit the website to find out more.

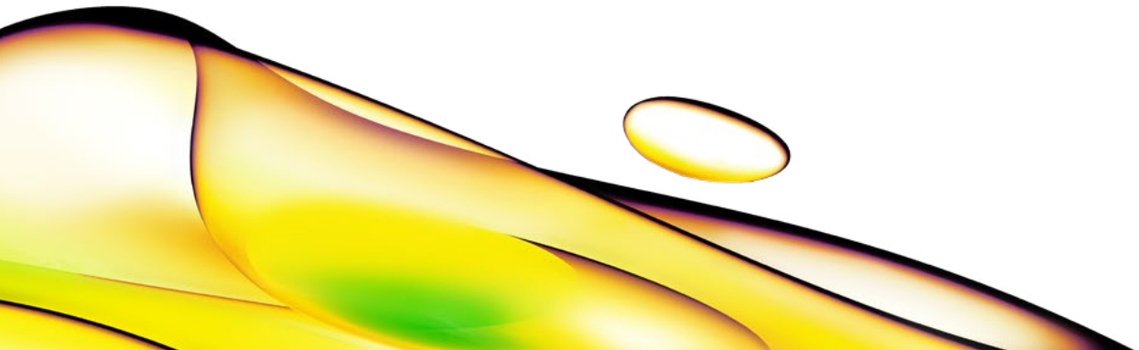




LabChip® cfDNA Assay on LabChip® GX Touch™ Nucleic Acid Analyzer

The LabChip cfDNA assay is a robust, high-throughput, automated, and easy-to-use assay that delivers digital assessment of cfDNA concentration and provides qualitative information about contaminants in the cfDNA sample.

- Improved cfDNA analysis from incorporation of 50 bp DNA internal standard.
- Able to detect cfDNA at concentrations as low as 25 pg/nL
- Ability to process up to 96 cfDNA samples in one chip preparation in less than 1.5 h



Bio-Rad Droplet Digital PCR

ddPCR can robustly detect low-abundance nucleic acids in blood samples while also obtaining critical information regarding quantifiable levels of mutated DNA[4]. This allows for the analysis of key biomarkers in cfDNA to measure the success of therapies and monitor for post-remission relapse early, when interventions might be most effective.

- Unmatched precision and reproducibility with a sensitivity down to 0.01% Variant Allele Frequency (VAF).
- Advanced multiplexing allows scientists to clearly discriminate and quantify up to a dozen biomarkers per well.
- Same-day turnaround time, simple workflows, and intuitive data

Digital PCR Assays and Kits

ddPCR mutation detection kits, wet-lab, and in-silico validated mutation detection assays capable of detecting mutant DNA present down to levels of 0.01% VAF.

- Suitable for use with circulating tumor DNA, liquid biopsy, and FFPE and other tissue samples.
- Over 4 million mutations, listed in the COSMIC database, assays designed instantly for targets and sequences for which validated assays are not available[5].
- Kits including positive and internal controls.

Explore all available assays for ddPCR analysis here.

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"We use cfDNA extracted with chemagic™ technology in droplet digital PCR (ddPCR) assays to support research of colorectal cancer, lung cancer and anal cancer (based on HPV cfDNA detection) with a sample to result turnaround time down to 24 h."

Professor Niels Pallisgaard, Dept of Pathology, Zealand University Hospital, Denmark

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DCC ctDNA ResearchCenter

Field Application – Clinical Research Highlight

At the Zealand University Hospital, the group of Prof. Niels Pallisgaard processes up to hundreds of plasma samples per month coming from the Nordic countries. Performing the full workflow from extraction to analysis with a sample to result turnaround time down to 24 h, the group has established consistent results in ctDNA analysis for their clinical research of diverse cancers. The extraction of cfDNA from 5 ml plasma is performed using the chemagic cfDNA 5k Kit H24 (CMG-1304) kit on the chemagic 360 instrument, and Bio-Rad ddPCR is performed for downstream detection of ctDNA. In addition, several sample quality controls and assay controls are incorporated to ensure accuracy and reliability of the workflow.

Their research has found that ctDNA monitoring in lung cancer can lead to earlier detection of treatment failure and clarified the majority of nonconclusive radiologic evaluations[6]. In squamous cell carcinoma of the anus, plasma HPV ctDNA assessed during chemo radiotherapy was able to divide subjects with anal cancer into three groups with significantly different risk of failure[7]. They also found that the ctDNA guided approach in metastatic colorectal cancer may potentially optimize adjuvant treatment, improving survival and reducing chemotherapy-related toxicity[8].

Watch webinar for more insights

To learn about Pallisgaard's work and valuable insights on cfDNA analysis, register for the webinar – **"Considerations for cfDNA isolation and dPCR analysis from Liquid Biopsies"**

Register for Webinar

Product/Ordering Information

Provider	Product name	Workflow Step	Product Number
Revvity	chemagic cfDNA 2K H24 Kit	Automated cfDNA extraction from 1 - 2 ml plasma	CMG-1302
Revvity	chemagic cfDNA 5K H24 Kit	Automated cfDNA extraction from 3 -5 ml plasma	CMG-1304
Revvity	chemagic cfDNA 10K H24 Kit	Automated cfDNA extraction from 6 - 10 ml plasma	CMG-1310
Revvity	chemagic cfDNA 18k Kit H12 Kit	Automated cfDNA extraction from 5 - 18 ml plasma	CMG-1318
Revvity	chemagic 360 instrument	Automated cfDNA extraction	2024-0020
Revvity	chemagic 360 24 Rod Head Set	Automated cfDNA extraction from up to 10 ml plasma	CMG-376
Revvity	chemagic 360 12 Rod Head Set	Automated cfDNA extraction from up to 18 ml plasma	CMG-371
Revvity	chemagic 13ml double-bottom rack H24	Automated cfDNA extraction with 24 Rod Head System (3 qty required)	CMG-13005340
Revvity	chemagic cfDNA 5K Kit	Manual cfDNA extraction from up to 5 ml plasma	CMG-134
Revvity	chemagic Stand F	Manual cfDNA extraction	CMG-302
Revvity	chemagic 2x12 Stand	Manual cfDNA extraction	CMG-300
Revvity	chemagic cfDNA Stand 12	Manual cfDNA extraction (optional)	CMG-306
Revvity	Mimix Multiplex I cfDNA in Synthetic Matrix II	Spike-in Reference material	HD917
Revvity	LabChip cfDNA Assay	cfDNA analysis and QC	CLS157242
Revvity	LabChip GX Touch 24 Nucleic Acid Analyzer	cfDNA analysis and QC for up to 48 samples	CLS138162
Revvity	LabChip GX Touch HT Nucleic Acid Analyzer	cfDNA analysis and QC for up to 384 samples	CLS137031
Bio-Rad	QX600 Droplet Digital PCR System	Quantification of key biomarkers in cfDNA samples	17007769
Bio-Rad	QX600 AutoDG Droplet Digital PCR System	Quantification of key biomarkers in cfDNA samples	17008371
Bio-Rad	QX600 Droplet Reader	Quantification of key biomarkers in cfDNA samples	12013328

References

1. Technical Note: Automated Circulating Cell-Free DNA Purification with the chemagic™ 360 Instrument.

2. cfDNA extraction with Horizon reference standard

3. Allelic Frequency Measurement of Multiplex I cfDNA Reference Standard Set using Droplet Digital PCR, Ion Torrent and MiSeq.

4. Application Note: Mutation Detection Multiplexing Using the QX600 Droplet Digital PCR System

5. Application Note: Rare Mutation Detection Best Practices Guidelines

6. Frank, M. S., Andersen, C. S. A., Ahlborn, L. B., Pallisgaard, N., Bodtger, U., & Gehl, J. Circulating Tumor DNA Monitoring Reveals Molecular Progression before Radiologic Progression in a Real-life Cohort of Patients with Advanced Non-small Cell Lung Cancer. Cancer Research Communications, 2022; 2(10), 1174–1187.

7. Lefèvre AC, Pallisgaard N, Kronborg C, Wind KL, Krag SRP, Spindler KG. The Clinical Value of Measuring Circulating HPV DNA during Chemo-Radiotherapy in Squamous Cell Carcinoma of the Anus. Cancers (Basel). 2021 May 18;13(10):2451.

8. Callesen LB, Hansen TF, Andersen RF, Pallisgaard N, Kramer S, Schlander S, Rafaelsen SR, Boysen AK, Jensen LH, Jakobsen A, Spindler KG. OPTIMISE: Optimisation of treatment selection and follow-up in oligometastatic colorectal cancer - a ctDNA-guided phase II randomised approach. Study protocol. Acta Oncol. 2022 Sep;61(9):1152-1156.

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