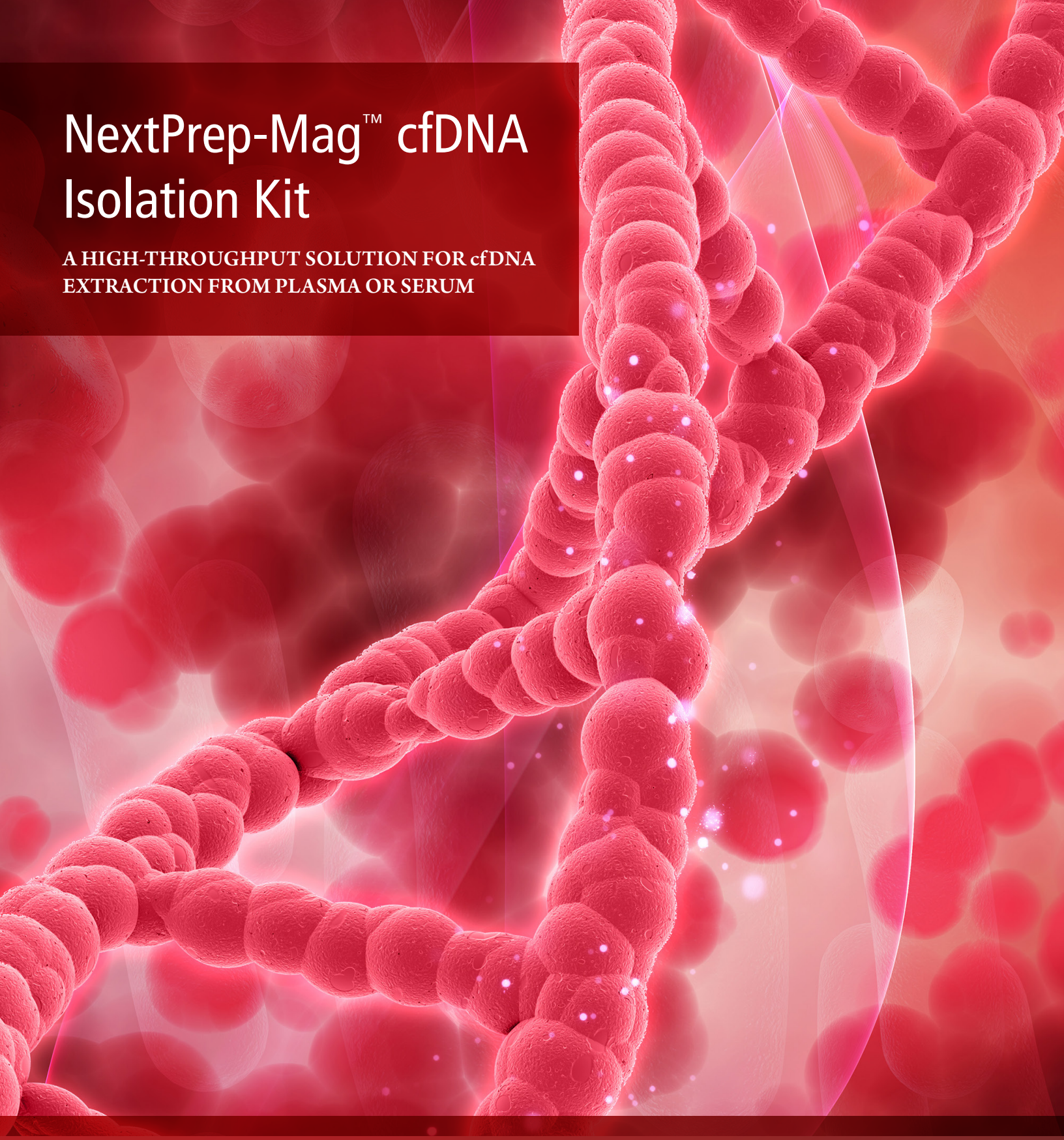
NextPrep-Mag™ cfDNA Isolation Kit

A HIGH-THROUGHPUT SOLUTION FOR cfDNA EXTRACTION FROM PLASMA OR SERUM



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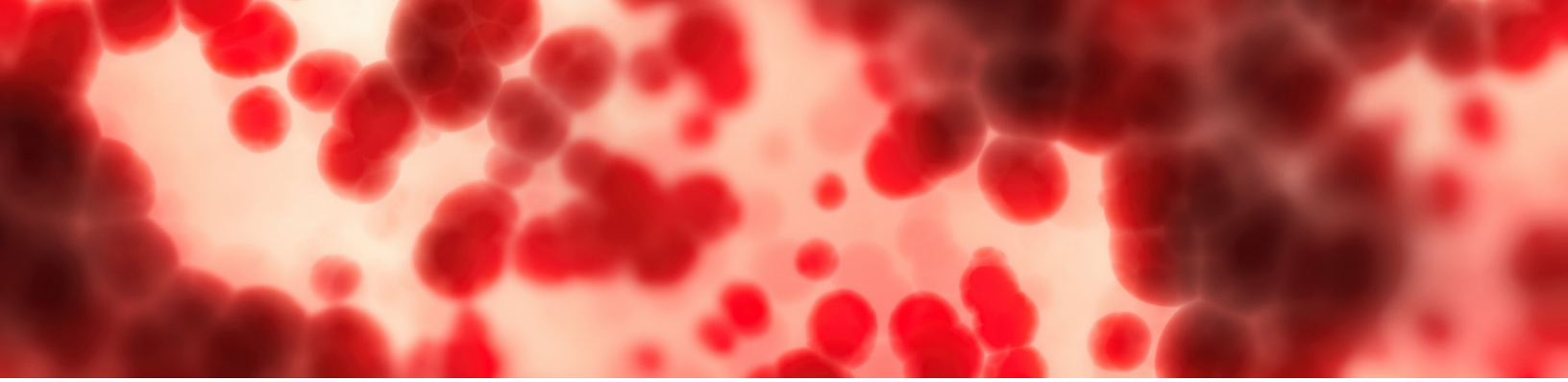


Significant research effort is being focused on developing clinical research reagents for prenatal disorders and malignant disease monitoring using DNA recovered from the cell-free fraction of blood. This has led to a need for more efficient methods for extracting cell-free DNA (cfDNA) from plasma or serum. To meet this need, PerkinElmer has developed an automated workflow for extracting cfDNA and subsequent next-generation sequencing. The new NextPrep-Mag™ cfDNA automated isolation kit offers many benefits.

- Streamlined extraction process
  - » Combined protease digestion and DNA binding step
  - » No post-extraction centrifugation steps
  - » No vacuum manifolds or column extender requirements
  - » Rapid attraction and dispersion of magnetic beads
  - » All steps carried out at room temperature
- High yields of pure cfDNA
- Compatibility with the chemagic™ 360, chemagic™ Prime™, and chemagic™ MSM I robotic platforms
- Compatibility of resulting cfDNA with automated NEXTFLEX® cell free DNA-seq kit workflow on the Sciclone® and Zephyr® G3 NGS workstations

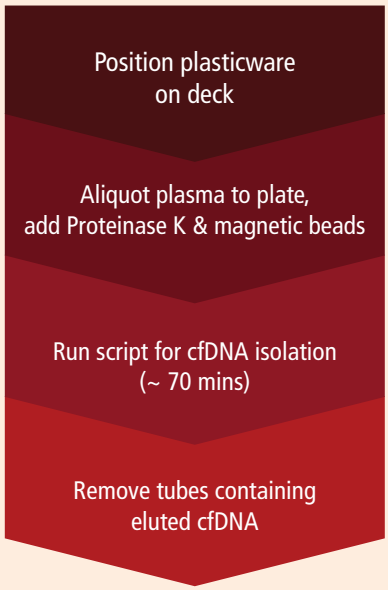
A

B

C

Figure 1. Automated Processing Portfolio. NextPrep-Mag™ cfDNA automated isolation kit (A), chemagic™ 360 instrument (B), and chemagic™ MSMI robotic platform (C).

A



B

Attributes	NextPrep-Mag™ cfDNA Automated Isolation Kit
Turnaround time	1 hr 15 mins
Hands-on time	15 mins
Isolation method	Magnetic beads
Automated extraction platforms	chemagic™ 360, chemagic™ MSM I & chemagic™ Prime™ instruments
Automated library prep platform	Sciclone® G3 NGS and Zephyr® G3 NGS workstations
Number of heating steps	None
Number of steps on ice	None
Carrier RNA requirement	No
User-supplied alcohol requirement	No, included in kit
Plasma input	5 mL on a 24-rod head system, 1.5 mL on a 96-rod head system*

* Protocol may be adjusted to accept lower and higher plasma input volumes. Please inquire.

Figure 2. Workflow. Protocol overview (A) and attributes (B)

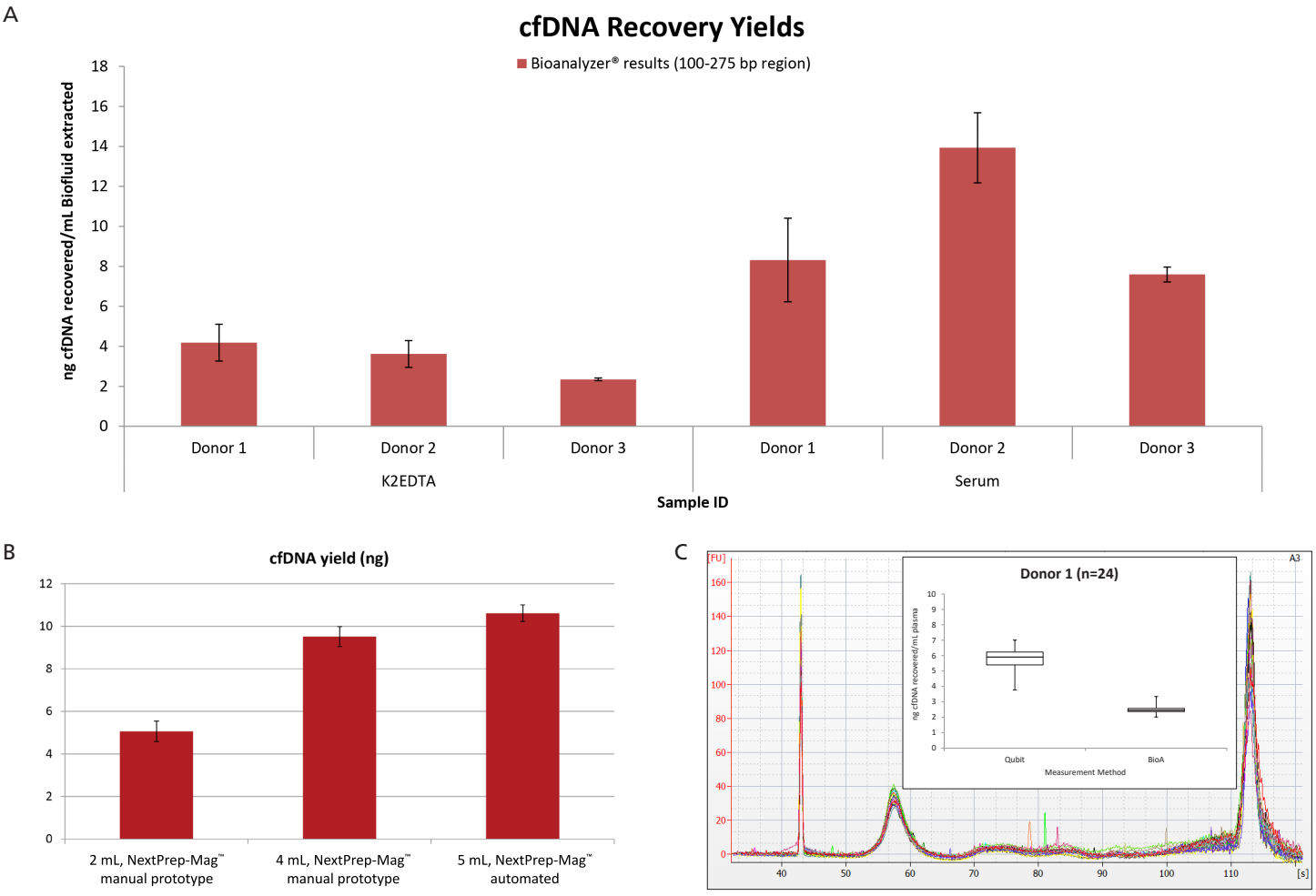
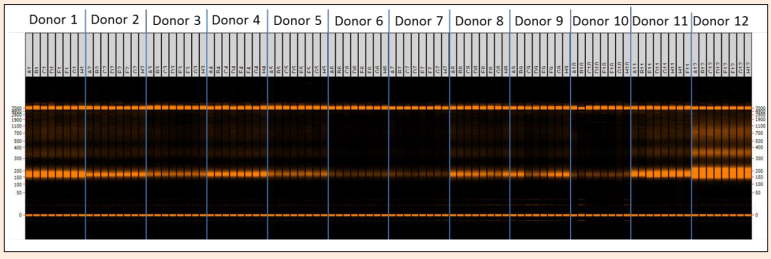
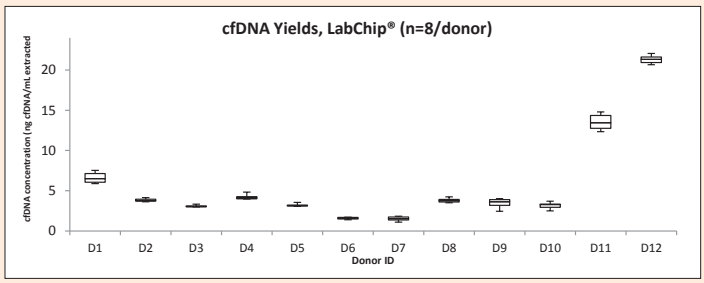


Figure 3. Analysis of cfDNA extracted with NextPrep-Mag™ cfDNA isolation kit on the chemagic™ 360 instrument on a 24-rod head system. *(A) 5 mL EDTA plasma or serum were extracted in duplicate and on two separate days using the NextPrep-Mag™ kit on the chemagic™ 360 instrument. Yields for the ~170 bp mononucleosome cfDNA fraction were determined by the 2100 Bioanalyzer® platform (Agilent®). (B) Plasma was extracted using the NextPrep-Mag™ manual kit with differing inputs and compared to the automated kit for yield. Results show that yield of cfDNA is scalable to sample input. (C) cfDNA was extracted from doubly-spun EDTA plasma in a single automated run (24 X 5 mL replicates). Reproducible yields are seen qualitatively by electropherogram overlay and quantitatively for both total DNA recovered (Qubit® dsDNA HS assay (Thermo Fisher Scientific®)) and for mononucleosome peak (2100 Bioanalyzer® platform (Agilent®),100-275 bp region). %CV's were 12% and 10%, respectively, for the two measurement methods.*





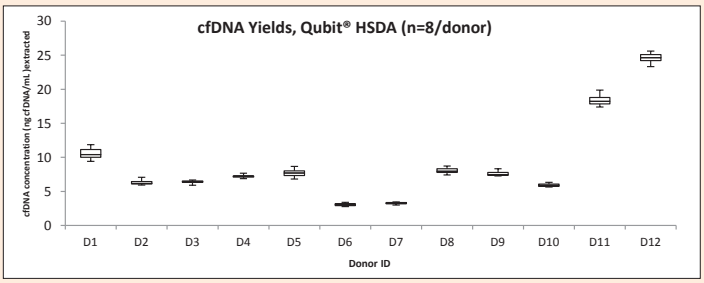


Figure 4.Figure 4: NextPrep-Mag™ cfDNA Automated Isolation kit for 1.5mL plasma/serum extraction is reliable and reproducible on the chemagic™ 360 instrument using the 96-rod head system. *Blood from Donors D1 to D12 were collected in K2EDTA tubes while Donors 11 and 12 were collected in Serum Separating Tubes (SST). The Labchip® GXII Touch™ HT instrument gel image of cfDNA extracted from all 96 wells in a single run is shown, along with box and whisker plots of cfDNA yields quantified on the Qubit® assay.*

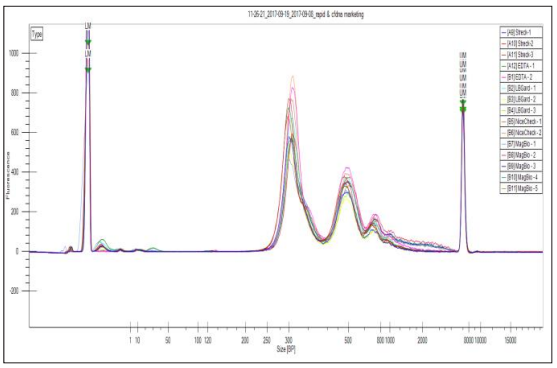
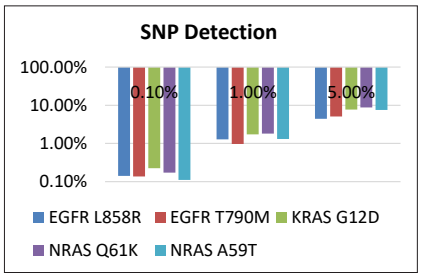


Figure 5. NGS library prep from cfDNA extracted using the NextPrep-Mag™ cfDNA Automated Isolation kit is reproducible across various collection methods. To further investigate reproducibility across various collection methods, plasma pooled from blood collected in five kinds of blood collection tubes (BD Vacutainer® EDTA tubes, Streck BCT® tubes, Biomatrica LBgard® tubes, NICE® Check tubes, MagBio® tubes) were used to extract cfDNA on the chemagic™ 360. For each tube type, the NEXTFLEX® cell free DNA-seq kit was used for the automated preparation of 15 whole genome libraries on the Sciclone® G3 NGS system using 10 cycles of PCR and diluted 1:10 for analysis on a PerkinElmer LabChip® HS DNA chip. (A) Overlaid traces from the 15 libraries with corresponding peaks from the various nucleosome-derived cfDNA populations are shown. An optional automated size-selection step can be included to recover only mononucleosome-derived library products. Results show robust library generation from all collection methods.

A



B

Expected Mutation Frequency			
Mutations	0.10%	1.0%	5.0%
Observed Mutation Frequency			
EGFR L858R	0.14%	1.28%	4.43%
EGFR T790M	0.14%	0.97%	5.11%
KRAS G12D	0.23%	1.74%	7.70%
NRAS Q61K	0.17%	1.81%	8.77%
NRAS A59T	0.11%	1.31%	7.56%

Figure 6. NGS analysis of cancer-associated variants in cfDNA standards manually extracted from synthetic plasma using the same NextPrep-Mag™ cfDNA Automated Isolation kit chemistry show concordance between the expected and observed allele frequencies determined by sequencing. (A, B) Horizon Discovery® cfDNA Standards, which include mutations in several cancer-associated genes present at 5%, 1%, and 0.1%, were extracted manually (due to low sample availability of synthetic plasma) using the NextPrep-Mag™ cfDNA automated kit chemistry. A commercially available kit targeting the cancer mutations in EGFR and KRAS/NRAS was used to prepare amplicon libraries for sequencing. Results show excellent agreement between the expected and observed allele frequencies determined by sequencing, which indicates extraction reagent compatibility with downstream molecular applications such as NGS.

ORDERING INFORMATION

CATALOG #	PRODUCT NAME	QUANTITY
NOVA-3825-05	NextPrep-Mag™ cfDNA Automated Isolation Kit (5 mL)	240 Isolations
NOVA-3825-06	NextPrep-Mag™ cfDNA Automated Isolation Kit (5 mL)	48 Isolations
NOVA-3825-10	NextPrep-Mag™ cfDNA Automated Isolation Kit (1.5 mL)	960 Isolations
	RELATED PRODUCTS	
NOVA-3825-01	NextPrep-Mag™ cfDNA Isolation Kit (< 1 mL - 3 mL)	16 - 50 isolations
NOVA-3825-03	NextPrep-Mag™ cfDNA Isolation Kit (3 mL – 5 mL)	50 isolations
NOVA-5150-01	NEXTFLEX® Cell Free DNA-Seq Kit for Illumina® Library Prep	8 rxns
NOVA-5150-02	NEXTFLEX® Cell Free DNA-Seq Kit for Illumina® Library Prep	48 rxns
NOVA-4002-01	NEXTFLEX® Cell Free DNA-Seq Kit for Ion Torrent™ Library Prep	8 rxns
NOVA-4002-02	NEXTFLEX® Cell Free DNA-Seq Kit for Ion Torrent™ Library Prep	48 rxns

For more information, please visit www.BiooScientific.com/cfDNA

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