

chemagic cfDNA extraction kit for HD917 cfDNA reference standard.

Introduction

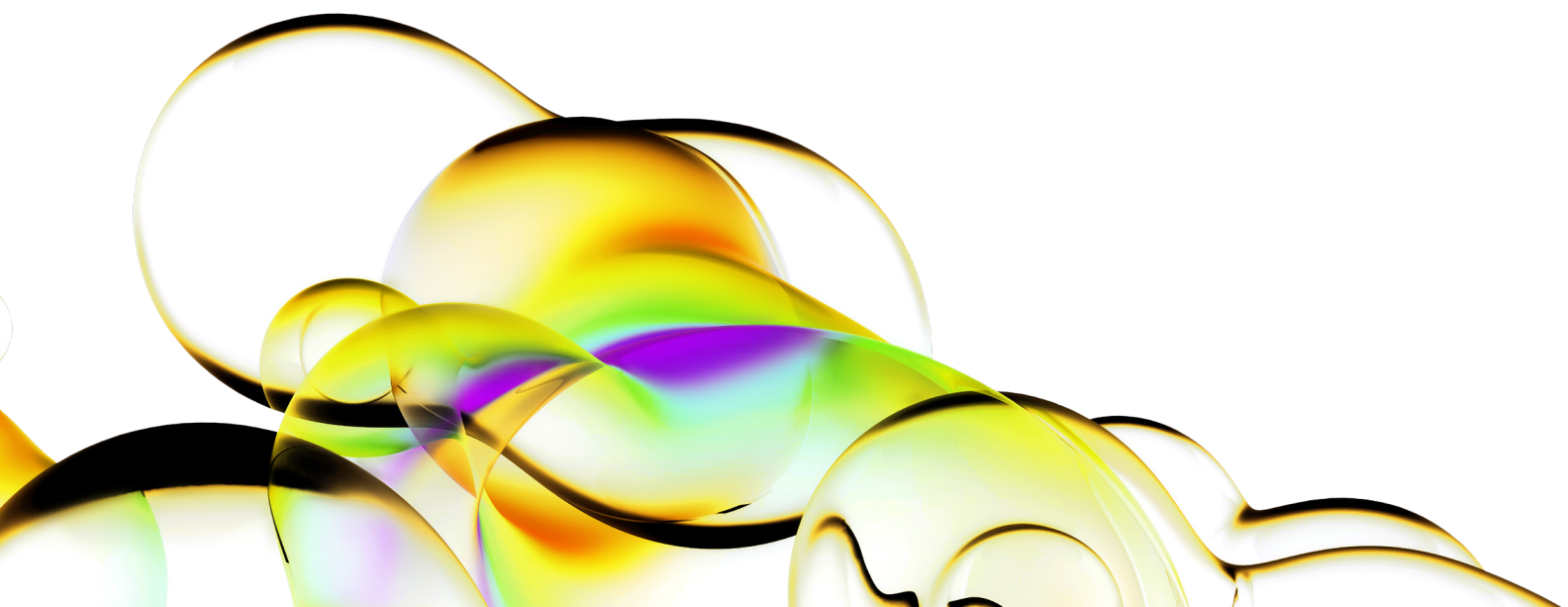
The analysis of cell free DNA (cfDNA) in human plasma is a noninvasive method in molecular diagnostics for detection of genetic alterations. It serves as an important biomarker in the field of oncology. Revvity develops reference standards consisting of fragmented DNA with genetically defined common cancer mutations spiked in synthetic plasma, providing reliable controls or liquid biopsy assays. These reference materials have a known range of allelic frequencies suitable for end-to-end process control. The aim of this study is to evaluate the compatibility between Revvity Mimix HD917 cfDNA reference standard with chemagic™ cfDNA extraction kit (CMG-134).

Product description

HD917 is a cell line-derived multiplex cfDNA reference standard spiked in a synthetic plasma. It is composed of the individual components covering multiple engineered single nucleotide variants (SNVs/SNPs) with 8 onco-relevant mutations at low allelic frequencies, ranging from 5% to as low as 0.1% (Table 1).

Table 1: HD917 cfDNA reference standard

Catalogue no	Description	SNB
HD912	5% multiplex I	50682
HD913	1% multiplex I	50683
HD914	0.1% multiplex I	50684
HD915	100% multiplex I wild type	50685



cfDNA extraction method

The majority of cfDNA extraction kits involve either column-based or magnetic bead-based DNA purification. Both involve the adsorption and elution of DNA molecules from a solid surface, typically silica or carboxyl-coated. The use of magnetic beads is often preferred for automated systems as they are easier to manipulate and eliminate the need for centrifugation. Many kits share the same underlying chemistry, but the efficiency extractions and size profiles of eluted DNA have been shown to vary.

Revvity cfDNA extraction kits are based on chemagic Technology using M-PVA Magnetic beads to ensure a high quality of nucleic acid purification. When compared to manual spin column methods, the manual magnetic bead-based kit showed improved yields as well as efficient removal of contaminants and exclusion of cross-contamination.

Results

Extraction efficiency

An input of 2 mL of HD917 was extracted by two different cfDNA extraction methods to compare extraction efficiencies. As shown in Table 2, improved yields were obtained with the new chemagic cfDNA 5k kit (CMG-134) when compared to our current column/bead-based extraction method.

Table 2: Comparison of extraction yield (%) of Column/bead based and chemagic cfDNA 5k kit (CMG-134) extraction platforms

Catalogue no	Column/bed	chemagic cfDNA 5k
HD912	67.00%	74.00%
HD913	66.86%	78.00%
HD914	63.23%	75.00%
HD915	58.04%	69.00%

Fragment size analysis and genetic composition

Similar length profiles to that of the current extraction method were obtained by TapeStation D1000 (Figure 1). In addition, the genetic composition of the selected sample was expressed as allelic frequency (%) by ddPCR, as shown in Table 3.

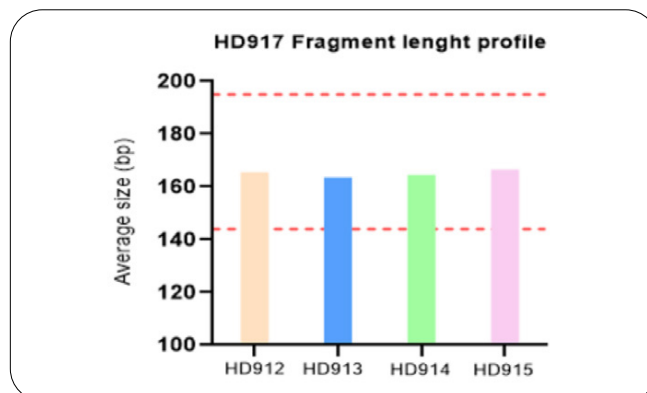


Figure 1: Fragment length profiles of HD917

Table 3: Genetic composition of HD912

Gene	Mutation	Expected AF (%)	Acceptance criteria		Measured AF (%)
			Low	High	
EGFR	T790M	5.00	3.50	6.50	4.70
NRAS	Q61K	6.30	4.40	8.20	6.00
PIK3CA	E545K	6.30	4.40	8.20	6.30

Conclusion

This study showcases how products within the Revvity life sciences group can be used in conjunction to support oncology NGS pipelines. The results confirm the compatibility of Revvity's HD917 with the chemagic cfDNA 5k kit. The magnetic bead method for cfDNA extraction enables labs to obtain increased DNA yield with no impact to downstream QC processes as the cfDNA fragment profile and allelic frequencies are maintained. HD917 is a highly compatible cell-based reference standard for cfDNA recovery and is compatible with the most commonly used extraction kits.

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