

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. Horizon 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 Horizon Discovery 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).

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

Table 1: HD917 cfDNA reference standard

Full product details of HD917: <https://horizondiscovery.com/en/reference-standards/products/multiplex-i-cfdna-in-synthetic-matrix-ii?catalognumber=HD917>

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.

PerkinElmer chemagen 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.

Find out more information on <https://chemagen.com/workflows/cfdna-isolation-from-plasma>

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.

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

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

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.

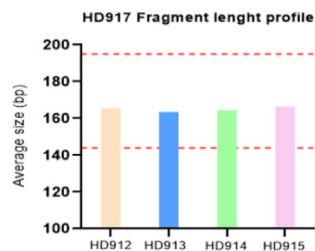


Figure 1: Fragment length profiles of HD917

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

Table 3: Genetic composition of HD912

Conclusion

This study showcases how products within the PerkinElmer life sciences group can be used in conjunction to support oncology NGS pipelines. The results confirm the compatibility of Horizon'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.

For more information

To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us

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