Myeloid Cancer Panel cfDNA (HD838) and Myeloid Cancer Panel Negative Control (HD839) Reference Standards.

Cell-free DNA control material, containing 15 clinically-relevant cell- line derived variants involved in the treatment and progression of Myeloid Cancers

Introduction

Revvity's Mimix™ Myeloid Cancer Panel cfDNA and Negative Control cell free DNA (cfDNA) Reference Standard is a highly characterized, clinically relevant, cell derived quality control material designed for use in Minimal Residual Disease (MRD).

It is a cancer-specific liquid biopsy reference standard which can be used to validate the molecular tests critical for diagnosis, treatment and monitoring of patient response.

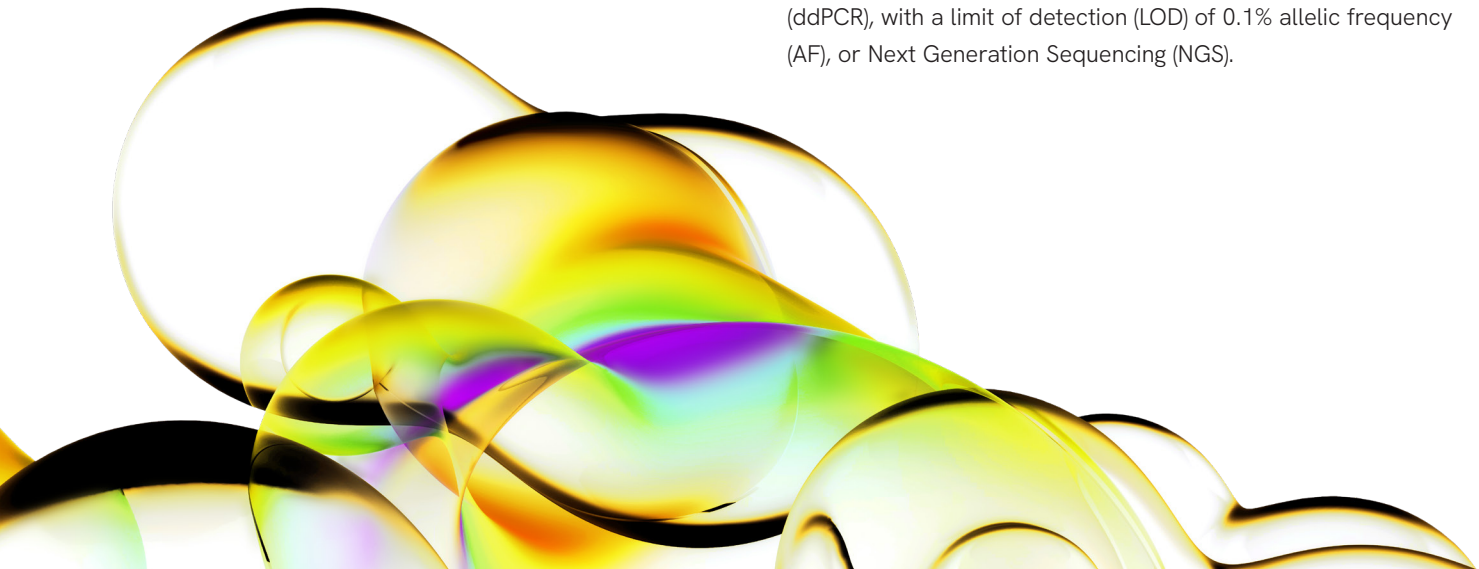
There is a strong need for high quality, well-characterized and reproducible cfDNA controls. Revvity has developed a new myeloid cancer specific multiplex panel which closely mimics clinical patient samples.

Background and product overview

Myeloid malignancies are a heterogeneous group of blood cancers caused by genetic or epigenetic alterations of the progenitor or hematopoietic stem cells. One of the most common disorders are the myeloid leukemia that results in the alteration of key immune responses specially against bacterial infection.

Leukemia is one of the most prevalent cancers globally and the tenth biggest cancer killer worldwide. It is also the most common type of childhood cancer under the age of 15.

Minimal or Measurable Residual Disease (MRD) is an important monitoring parameter that refers to a chemotherapy/ radiotherapy- surviving leukemia cell population that gives rise to relapse of the disease. The detection of MRD is critical for predicting the outcome and for selecting the intensity of further treatment strategies, therefore, MRD can only be detected through highly sensitive methods, such as droplet digital PCR (ddPCR), with a limit of detection (LOD) of 0.1% allelic frequency (AF), or Next Generation Sequencing (NGS).



Revvity has developed this new cfDNA version of the HD829 gDNA Myeloid Cancer Reference Standard, which is comprised of a selected panel of variants at reduced AFs at ≤ 1% to comply with MRD market needs in cfDNA format matched with a wild type negative control.

This panel is highly characterized, cell-line-derived and reproducible, containing a blend of cell lines which include 15 clinically relevant mutations associated with myeloid cancers, involved in the survival outcome, progression, risk stratification and prediction of further treatment strategies (Table 1).

Product Highlights

- 15 multiplexed variants in 14 genes
- Cell-line-derived material which mimics clinical patient samples
- Low AF to challenge limit of detection
- Available Negative Control to reach even lower AF

Table 1: HD836 Prostate Panel variants

Gene	DNA sequence change	Amino acid change	Allelic frequencies (%)
ABL1	c.1001C>T	p.T315I	0.50
ASXL1	c.2388G>T	p.W796C	0.50
CBL	c.1208C>T	p.S403F	0.50
DNMT3A	c.2644C>T	p.R882C	0.50
EZH2	c.1253G>A	p.R418Q	0.50
FLT3	c.2503G>T	p.D835Y	0.50
IDH1	c.394C>T	p.R132C	0.50
IDH2	c.515G>A	p.R172K	0.50
JAK2	c.1611_1616del	p.F537-K539>L	0.50
JAK2	c.1849G>T	p.V617F	0.50
NPM1	c.860_863dup	p.W288Cfs*12	0.50
NRAS	c.182A>T	p.Q61L	1.00
SF3B1	c.2219G>A	p.G740E	0.50
TET2	c.3782G>A	p.R1261H	0.50
TP53	c.722C>T	p.S241F	0.50

Product development

Cell lines containing variants clinically relevant to myeloid cancers and cells genetically engineered for desired variants at specific genomic locations were selected from HD829. All cell lines were validated, tested and confirmed by Droplet Digital PCR (Figure 1).

The product underwent validation at different stages during the product development and manufacturing process to ensure the accuracy and consistency of Revvity's cfDNA Myeloid Cancer Panel and cfDNA Negative Control.

The genomic DNA was extracted from the cell lines and blended in defined ratios to yield a multiplex blend containing a range of mutations with allelic frequencies as low as 0.5%. In addition, the matching wild type control, which is negative for all variants found in the Myeloid Cancer Panel cfDNA reference standard, allows further VAF dilution to reach even lower allelic frequencies.

The blended gDNA is fragmented by mechanical shearing to 160bp and validated through precise quality control steps to assess the fragment length using the TapeStation D1000 system and concentration by Qubit dsDNA BR assay (Figure 2).

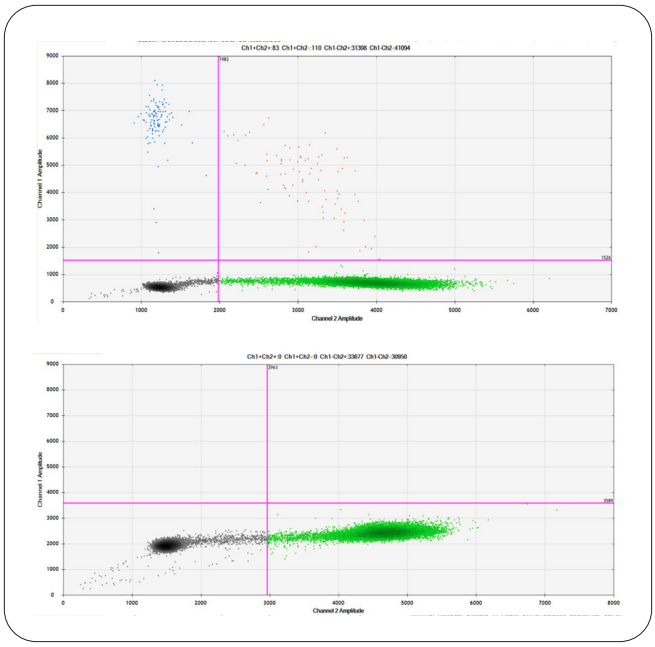


Figure 1. Representation of one biomarker of HD838 and HD839 analyzed by ddPCR. Respective allelic frequencies were assessed by quadruplicate Droplet Digital PCR analysis for each variant using specific probes on the Bio-Rad QX200 ddPCR platform. The ddPCR assays on the cfDNA fragments confirmed all the claimed variants at expected allelic frequencies (orange and blue dots) with high reproducibility.

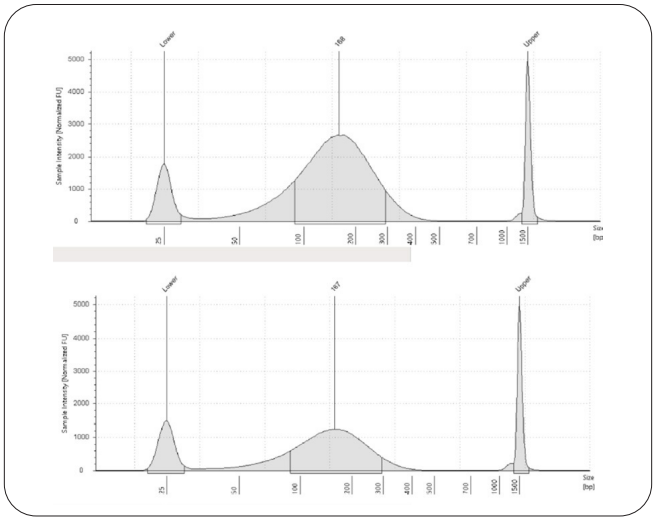


Figure 2. cfDNA analysis for size distribution by TapeStation D1000 system. Both HD838 and HD839 are within the acceptance criteria for average size of 160bp (144bp-176bp).

In this application note we show how we have developed and validated the Revvity's cfDNA Myeloid Cancer Panel and cfDNA Negative Control reference standard as a valuable control for achieving confidence in all the stages of development and validation of liquid biopsy assays. This reference standard can be used to validate and control the detection assays of genetic variants related to MRD myeloid cancers in patient samples like liquid biopsy and plasma.

Technical data

Format: cfDNA

Genes covered: ABL1, ASXL1, CBL, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, NPM1, NRAS, SF3B1, TET2, TP53
Unit size: 350 ng (20 ng/µl)

General information

Intended Use: For assay validation and routine monitoring of assay performance. Research use only. Not for use in diagnostic procedures.

Storage: 4°C
Expiry: See all product shelf-life information

References

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2. Stanojevic M, Grant M, Vesely SK, Knoblach S, Kanakry CG, Nazarian J, Panditharatna E, Panchapakesan K, Gress RE, Holter-Chakrabarty J, Williams KM. Peripheral blood marker of residual acute leukemia after hematopoietic cell transplantation using multi-plex digital droplet PCR. Front Immunol. 2022 Sep 29;13:999298.
3. Contreras Yametti GP, Ostrow TH, Jasinski S, Raetz EA, Carroll WL, Evensen NA. Minimal Residual Disease in Acute Lymphoblastic Leukemia: Current Practice and Future Directions. Cancers (Basel). 2021 Apr 13;13(8):1847.

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