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TECHNICAL NOTE

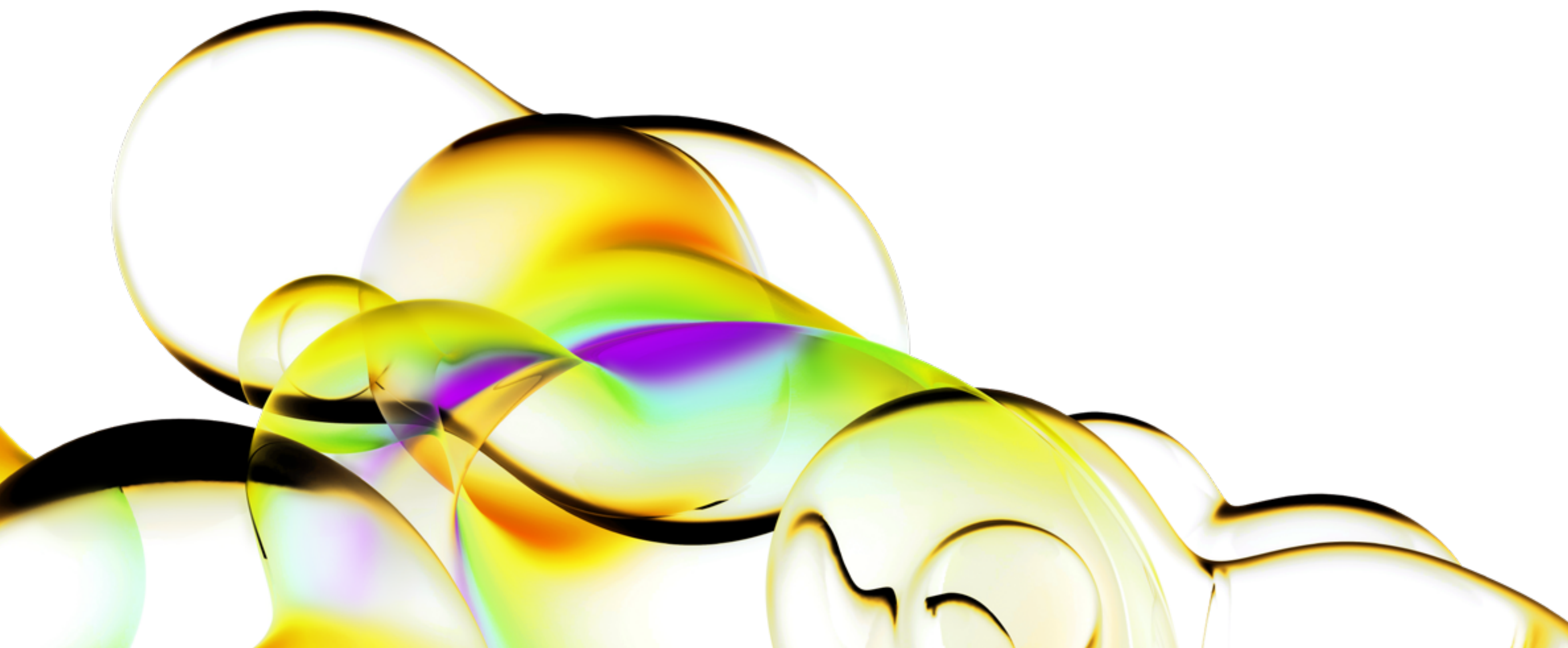
Non-small cell lung cancer ctDNA panel (HD844) and negative control (HD845) reference standards.

Circulating tumor DNA reference material, containing 22 clinically relevant cell-line derived variants involved in the development and progression of Non-Small Cell Lung Cancer (NSCLC).

Introduction

There is a strong need for high quality, well-characterized circulating tumor DNA (ctDNA) controls which closely mimic patient samples. Revvity has developed the Mimix™ Non-Small Cell Lung Cancer (NSCLC) Panel and Negative Control ctDNA Reference Standard which is a clinically relevant, cell-line derived, quality control material designed for use in Minimal Residual Disease (MRD) detection of NSCLC.

This 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.



Background and Product Overview

Non-small cell lung cancer is one of the most prevalent cancers worldwide, being the 2nd most common cancer in both men and women around the world. It represents the 1st leading cause of cancer deaths in both genders (18% of all cancer deaths), with more than 2.2 million people affected globally, accounting for 11.4% of all cancers *(Global Cancer Observatory, WHO 2020)*.

Non-small cell lung cancer remains an elusive target in liquid biopsy-based testing, with advanced cancers having emerging biomarkers as therapeutic targets. Several researchers have postulated the potential clinical value of ctDNA as a diagnostic tool for early lung cancer, due to its increased levels in NSCLC patients when compared with healthy individuals. ctDNA levels have been shown to have a specificity >95% and sensitivity >40% for distinguishing NSCLC patients from healthy individuals. It is important to consider that these values can be influenced by factors such as the choice of assay, the genetic characteristics of the tumor, and the prevalence of lung cancer in the study population. Additionally, some ctDNA assays may be optimized for early detection, while others may focus on monitoring treatment response or detecting recurrence. (Duffy MJ et al., CCLM 2021, Peng M et al., J. Med. Gen. 2021, Garcia J et al., Sci. Rep. 2021).

Minimal or Measurable Residual Disease (MRD) is an important monitoring parameter that refers to a chemotherapy/ radiotherapy-surviving cancer cell population that gives rise to relapse of the disease (Drahomír Kolenčík et al., *Cancers 2020*). 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 NGS. (Klaus Pantel et al., Nature 2019, Luskin MR et al., Nature 2018).

This new ctDNA NSCLC multiplexed panel reference standard, which is comprised of a selected panel of variants with reduced AFs at ≤1.20% to comply with MRD market needs matched with a negative control.

The NSCLC-associated variants are in the context of the complete genome, so the panel will perform similarly to a patient genome sample during molecular testing. This panel is highly characterized, cell-line-derived and reproducible, containing a blend of cell lines which include 22 clinically relevant mutations associated with NSCLC, involved in the survival outcome, progression, risk stratification and prediction of further treatment strategies (Tables 1 and 2).

Table 1: Normal function and clinical impact of genes included in HD844.

Gene	Normal Function	Clinical implications in NSCLC
EGFR	Receptor tyrosine kinase involved in many different cellular functions, prominently in cell proliferation.	Plays a crucial role in tumor cells proliferation and survival.
KRAS	Proto-oncogene encoding a GTPase protein involved in the RAS/MAPK signalling pathway.	Key gene in the Adenoma-Carcinoma sequence, promotes uncontrolled proliferation of cells.
BRAF	Proto-oncogene. Serine/Threonine protein kinase involved in epidermal growth factor receptor (EGFR) signalling that is important in cell proliferation, angiogenesis, cell migration, cell survival and cell adhesion.	V600E mutation is the most common BRAF mutation found in human cancers. Allows abnormal cells to grow and divide without external signals.
MET	Receptor tyrosine kinase regulating many different cellular processes including proliferation, scattering, morphogenesis and survival.	Deregulation in MET signalling can induce tumor invasion and metastasis and can interact with other signalling pathways such as EGFR.
ERBB2	Receptor tyrosine-protein kinase involved in growth and proliferation of the endothelium.	Amplification promotes tumorigenesis through deregulation of growth and proliferation signalling pathways.
PIK3CA	Encodes for the catalytic subunit of PI3K kinase protein. important for the regulation of cellular functions such as division, protein synthesis, angiogenesis.	Increased signalling contributes to the uncontrolled proliferation of cells, leading to cancer development.
NRAS	Proto-oncogene encoding a GTPase protein, which plays a role in signal transduction to the cell's nucleus.	Increased signalling contributes to the uncontrolled proliferation of cells, leading to cancer development.

Table 2: Variant information included in HD844.

Gene	DNA change	Amino acid change	AF (%)/CNV
BRAF	c.1799T>A	p.V600E	1.20
EGFR	c.2369C>T	p.T790M	0.80
EGFR	c.2156G>C	p.G719A	0.50
EGFR	c.2303G>T	p.S768I	0.80
EGFR	c.2582T>A	p.L861Q	0.80
EGFR	c.2573T>G	p.L858R	0.80
EGFR	c.2235_2249del	p.E746_A750del	0.80
EGFR	c.2236_2250del	p.E746_A750del	0.80
EGFR	c.2240_2257del	p.L747_P753delinsS	1.00
ERBB2	N/A	Amplification	6 copies
ERBB2	c.2313_2324dup	p.Y772_A775dup	0.30
ERBB2	c.2331_2339dup	p.G778_P780dup	0.30
KRAS	c.34G>T	p.G12C	1.00
KRAS	c.35G>T	p.G12V	0.50
KRAS	c.35G>A	p.G12D	1.00
MET	N/A	Amplification	4 copies
MET	c.3082+1G>T	Unknown (Exon 14 skipping)	0.80
NRAS	c.181C>A	p.Q61K	0.50
NRAS	c.182A>T	p.Q61L	0.50
NRAS	c.182A>G	p.Q61R	0.50
PIK3CA	c.1624G>A	p.E542K	1.00
PIK3CA	c.3140A>G	p.H1047R	0.80

** Alternate nomenclature c.2324_2325ins12 (p.A775_G776insYVMA) (Kris et. al., Ann Oncol 2015)

Product validation

Cell lines containing pathogenic somatic variants and cells genetically engineered for desired variants at specific genomic locations were chosen to cover the most clinically relevant pathways in Non-Small Cell Lung Cancer. All cell lines undergo validation which includes ploidy analysis and copy number determination for every gene of interest. All variants were confirmed by Sanger Sequencing and ddPCR. All AFs were validated, tested and confirmed by ddPCR (Figure 1), and can be found on the batch-specific Certificate of Analysis (CoA).

The product underwent validation at different stages during the product development and manufacturing process as per ISO 13485 to ensure the accuracy and consistency of Revvity's NSCLC ctDNA Panel and 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 0.30%. In addition, the matching negative control, which is negative for all variants found in the NSCLC ctDNA Panel reference standard, allows further VAF dilution to reach even lower allelic frequencies.

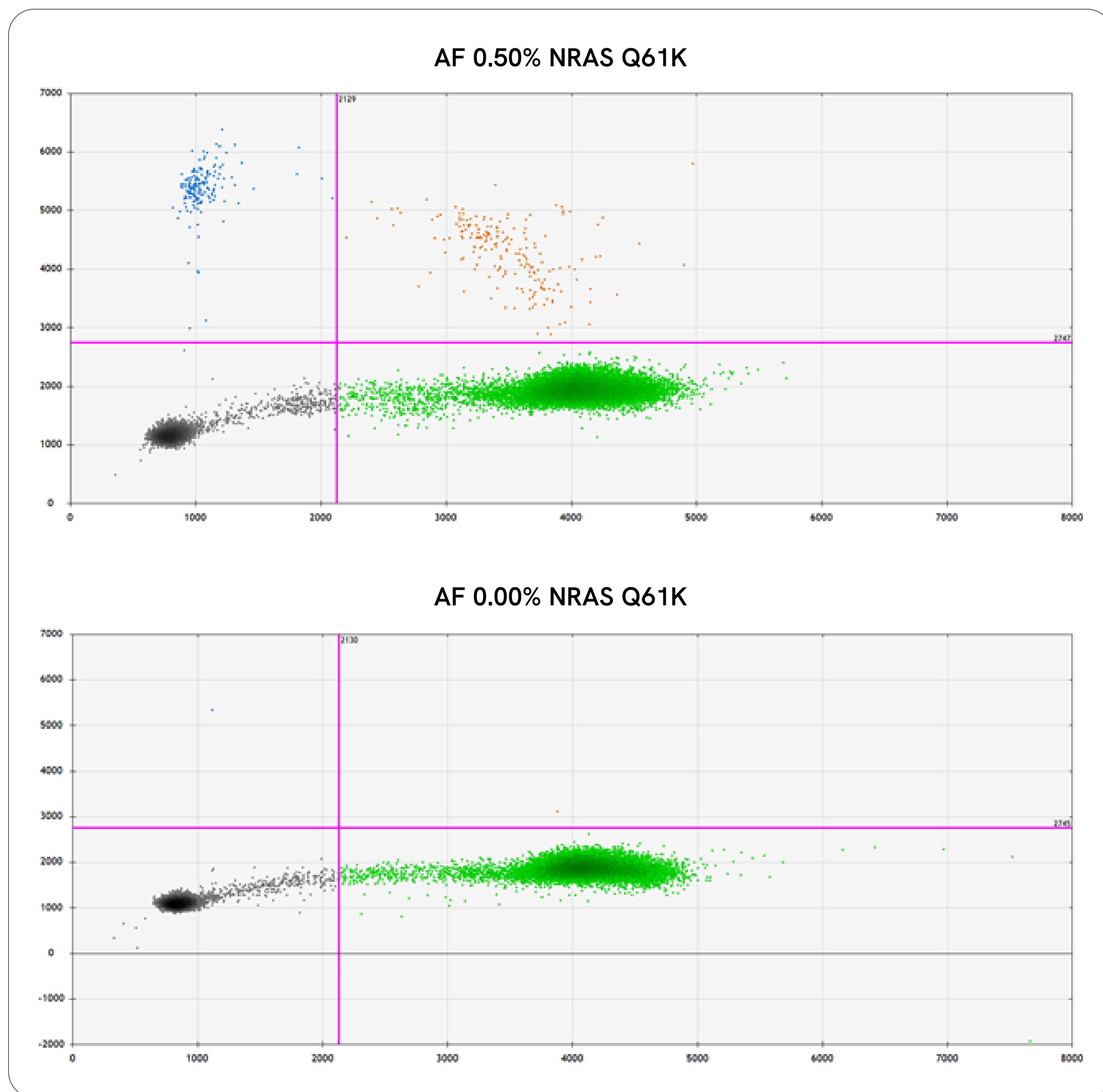


Figure 1: Representation of one biomarker of HD844 (top) and HD845 (bottom) analyzed by ddPCR. Respective allelic frequencies were assessed by quadruplicate Droplet Digital PCR analysis for each variant using specific probes on the Bio-Rad QX200 ddPCR platform. The ddPCR assays on the ctDNA fragments confirmed all the claimed variants at expected allelic frequencies (orange and blue dots) with high reproducibility.

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)*.

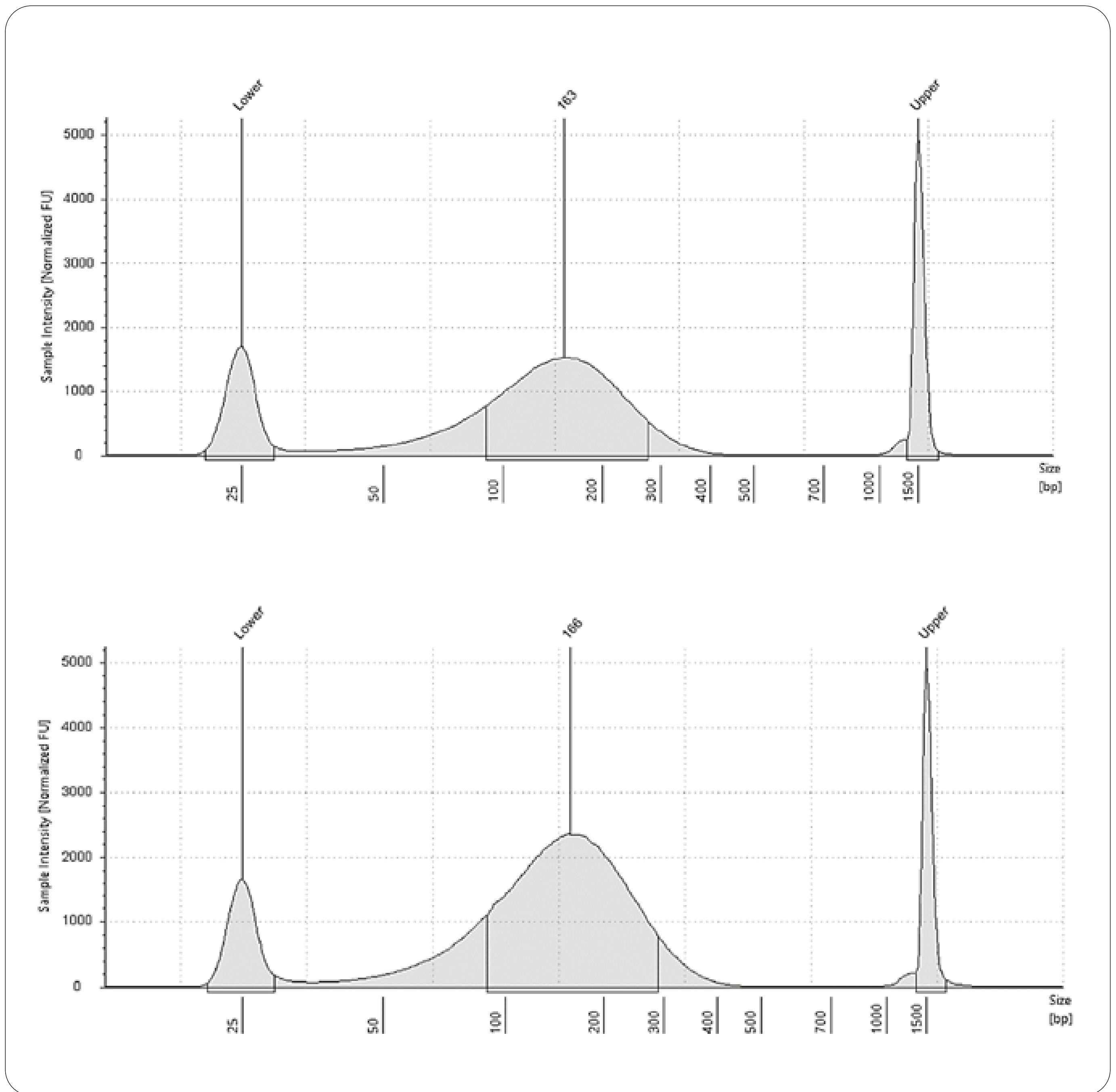


Figure 2: ctDNA analysis for size distribution by TapeStation D1000 system. Representative batches of HD844 (top) and HD845 (bottom) are within the acceptance criteria for an average size of ~160bp (144bp-176bp).

In this technical note we show how we have developed and validated the Revvity's NSCLC ctDNA Panel and Negative Control reference standard as a valuable control for achieving confidence at 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 NSCLC in patient samples like liquid biopsy and plasma.

Technical data

Format: ctDNA

Genes covered: *EGFR, KRAS, BRAF, MET, ERBB2, PIK3CA, NRAS*

Unit size: 350 ng (20 ng/µl)

Buffer: Tris-EDTA (10mM Tris-HCl, 1mM EDTA), pH 8.0

DNA extraction: Maxwell platform

Quality: Agilent TapeStation D1000 system

DNA quantification: Qubit dsDNA BR assay

Intended Use: for Assay developers, Molecular diagnostic labs (Research use only) and Proficiency testing.

General information

Storage: 4°C

Expiry: https://horizondiscovery.com/en/reference-standards/reference-standards-product-information

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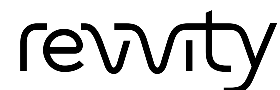
Product Highlights

- 22 multiplexed variants in 7 clinically relevant genes
- Cell-line-derived material which mimics clinical patient samples
- Low AF to challenge limit of detection
- Available matched Negative Control to reach even lower AF



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