Mimix™ Quantitative Multiplex FFPE Reference Standard

IVD

For In Vitro Diagnostic Use

REF HD200-IVD Mimix™ Quantitative Multiplex FFPE Reference Standard

Intended Use

The Mimix™ quantitative multiplex FFPE reference standard is a commutable control material comprising a formalin-fixed paraffin-embedded (FFPE) curl derived from human cancer cell lines, containing 11 variants across 6 cancer genes. It is intended for qualitative and/or quantitative monitoring next-generation sequencing (NGS) or droplet digital polymerase chain reaction (ddPCR) assays designed to detect somatic mutations in genomic DNA (gDNA) from human samples for *in vitro* diagnostic use.

The gDNA obtained from the Mimix quantitative multiplex FFPE reference standard can be used to monitor NGS or ddPCR workflow, test performance, assay variation, and helps identify increases in random or systematic errors. This product is for professional laboratory use only.

Summary and Explanation

NGS assays are widely adopted by researchers as well as clinical and diagnostic laboratories to analyze the genetic make-up of patient-derived samples, making it useful to gain insightful data on common or rare diseases[1]. Providing high-throughput data, NGS helps identify genetic differences in patients or any genetic changes introduced as certain diseases progress[2]. Similarly, digital PCR workflows, including ddPCR, are commonly used to enhance traditional PCR performance by allowing a significantly increased number of reactions to be performed and analyzed real time[3].

The Mimix™ Quantitative Multiplex FFPE Reference Standard enables direct analysis of patient-derived DNA samples against 11 cancer-related somatic mutations for fast and accurate results as part of diagnostic tests and scientific research. The product is designed to fit into NGS and ddPCR workflows to help accurately analyze patient-derived samples.

The Quantitative Multiplex FFPE standard is a curl mimicking patient tissue sections, sectioned from an FFPE block derived from well-established cell lines via cell pellet collection and blending followed by fixation in 4% paraformaldehyde (PFA) and paraffin embedding. Each FFPE block contains approximately $3x10^8$ cells, which corresponds to approximately 3.5 $x10^5$ cells per section. The reference standard contains 11 key cancer genes, at known allelic frequencies between 0.5% and 27%.

The Mimix™ Quantitative Multiplex FFPE Reference Standard is a multiplex standard covering mutations on the following genes: BRAF, KIT, EGFR, KRAS, NRAS, PIK3CA.

Principles of Operation

The Mimix™ Quantitative Multiplex FFPE Reference Standard contains a single curl suitable for gDNA extraction, derived directly from cell lines to mimic patient samples. No synthetic DNA is incorporated into this product. Every batch of Quantitative Multiplex FFPE has 11 variants confirmed by ddPCR (Table 1). The FFPE curl is intended to be integrated into the customer's workflow for DNA extraction, to monitor performance and systematic variation.

In addition to ddPCR, further quality control of this product is performed via agarose gel electrophoresis and fluorometric analysis.

Material Provided

Mimix™ Quantitative Multiplex FFPE Reference Standard

- Catalogue/Model number: HD200-IVD
  One vial, 1x FFPE curl, 15 µm thickness.

Table 1:Verified variants and the corresponding allelic frequencies (AF) confirmed by ddPCR.

Chromosome	Gene	Variant	Expected AF	Acceptance Range
chr7 (140453136)	BRAF	p.V600E	10.70%	7.49 - 13.91%
chr7 (55241707)	EGFR	p.G719S	24.50%	22.10 - 27.00%
chr7 (55249071)	EGFR	p.T790M	0.90%	0.60 - 1.20%
chr7 (55259515)	EGFR	p.L858R	2.80%	1.68 - 3.92%
chr7 (55242464)	EGFR	p.E746_A750 delELREA	1.90%	1.30 - 2.50%
chr4 (55599321)	KIT	p.D816V	10.00%	8.00 - 12.00%
chr12 (25398281)	KRAS	p.G13D	15.00%	12.00 - 18.00%
chr12 (25398284)	KRAS	p.G12D	6.30%	5.00 - 7.60%
chr1 (115256530)	NRAS	p.Q61K	12.50%	10.00 - 15.00%
chr3 (178936091)	PIK3CA	p.E545K	8.80%	7.00 - 10.60%
chr3 (178952085)	PIK3CA	p.H1047R	17.50%	14.00 - 21.00%

⚠ Precautions and Warnings

- This product is not classified as dangerous, however; users are advised to handle all materials as potentially biohazardous, following standard laboratory safety procedures.
- Dispose of all waste materials in accordance with local regulations.
- Avoid contamination of the product when opening and closing the vial by making sure that the curl is intact and sits at the bottom of the vial.
- Inspect the vial for any signs of melting before opening.

Storage and Handling

- Store unopened at 2 to 8 °C.
- Do not use this product beyond the expiration date printed on the product label.
- Please see the following instructions for handling:
  - Open the tube by twisting and lifting the lid.
  - If needed, please remove the curl carefully without destruction by using suitable forceps (e.g. anatomical forceps) and transfer the curl appropriately.
  - If the whole curl is to be removed from the vial, we recommend all handling takes place at temperatures below room temperature (~15-25 °C).
  - If the curl does not need to be transferred, please keep it in the vial and perform your assay accordingly (e.g. gDNA extraction).

Quality Control and Performance Characteristics

Variant detection when using the Mimix™ Quantitative Multiplex FFPE Standard may vary depending on the library preparation method, sequencing platform, and bioinformatics pipeline used for NGS assays, as well as primer/probe set, analysis type/platform, and assay conditions for ddPCR. The expected variant list of the OncoSpan FFPE provided can be used to compare each subsequent run.

Following our QC procedure, the acceptance criteria are described below (Table 2). Users should note that results will vary depending on the equipment and workflow used. Users are expected to perform their own validation to determine AFs within the workflow to be monitored. The below reported data was obtained using the validated equipment and workflow as specified for each quality control test.

Table 2: List of quality control tests applied and the corresponding acceptance criteria.

Test name	Test purpose	Acceptance criteria
Fluorometric analysis	Measure quantity and quality of FFPE DNA	dsDNA ≥400ng
ddPCR	Measure stability of AF	Within the accepted target range. Refer to Table 1.

The gDNA yield obtained from Quantitative Multiplex FFPE Reference Standard is shown in Figure 1.

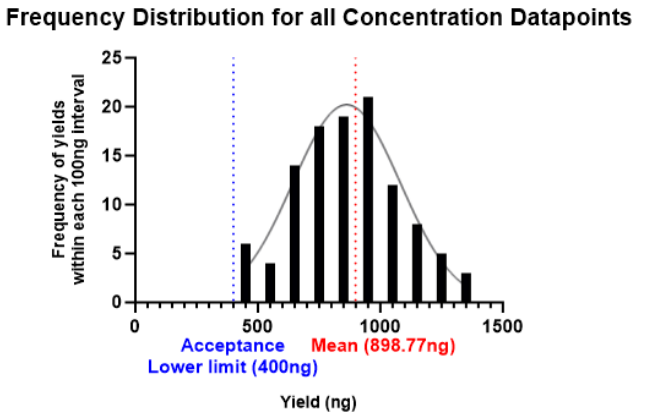

Figure 1. Frequency distribution for gDNA amount extracted from Quantitative Multiplex FFPE Reference Standard. The mean and lower acceptance criteria are shown.

Batch to batch variability of gDNA yield extracted from Quantitative Multiplex FFPE Reference Standard is shown in Figure 2.

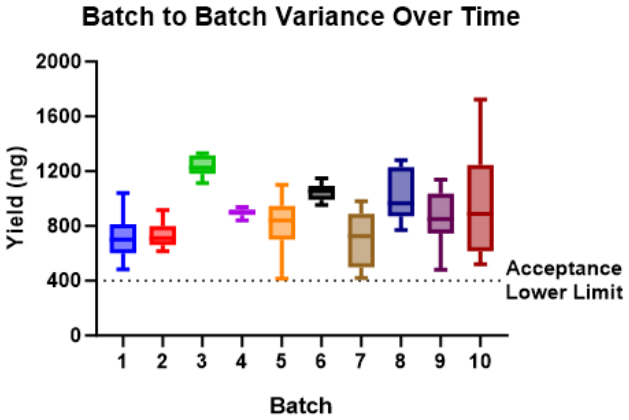

Figure 2. Batch-to-batch yield of gDNA extracted from Quantitative Multiplex FFPE Reference Standard.

Batch to batch variation in allelic frequencies of mutations tested for in the Quantitative Multiplex FFPE Reference Standard are shown in Figure 3.

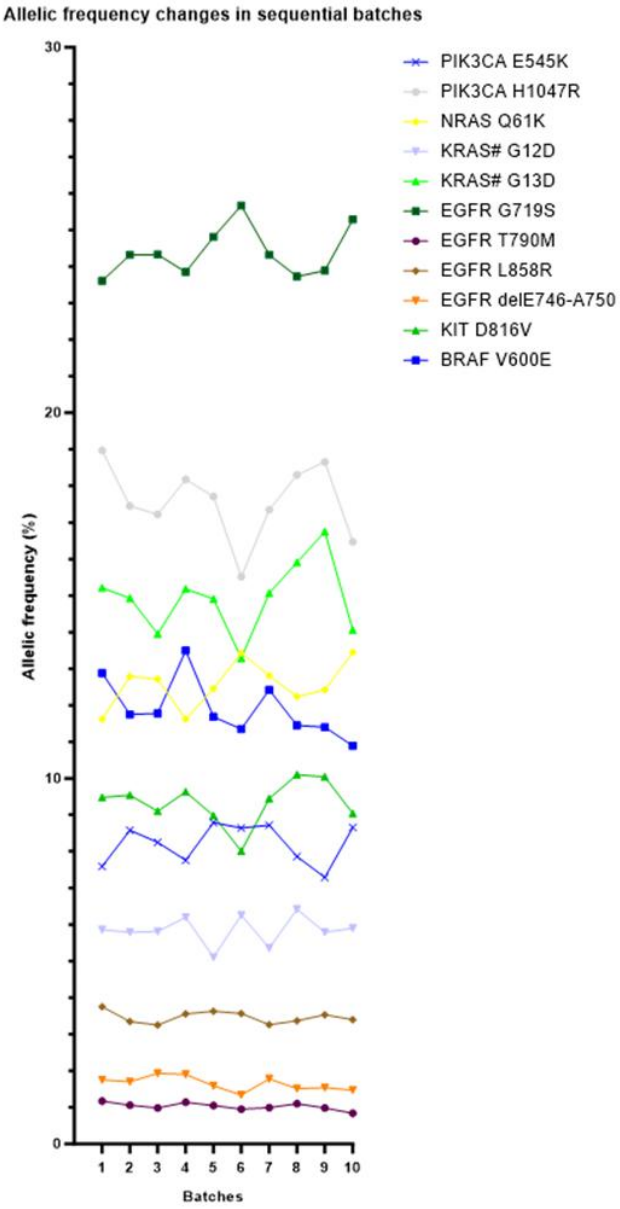

Figure 3. Batch to batch variation of AF for different genes and mutations in the Quantitative Multiplex FFPE Reference Standard confirmed by ddPCR.

Limitations

- The Mimix™ Quantitative Multiplex FFPE Reference Standard is not to be used as a substitute for the internal controls provided by manufacturers within their IVD assay kits.

Technical Support

Scientific support
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Customer support

Tel:800-235-9880 (Option 1) Or 303-604-9499 (Option 2)
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References

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3. Mirabile A, Sangiorgio G, Bonacci PG, et al. Advancing Pathogen Identification: The Role of Digital PCR in Enhancing Diagnostic Power in Different Settings. Diagnostics (Basel). 2024;14(15):1598. Published 2024 Jul 25. doi:10.3390/diagnostics14151598 Diagnostics (Basel). 2024;14(15):1598. Published 2024 Jul 25. doi:10.3390/diagnostics14151598

Glossary

IVD In Vitro Diagnostic Medical Device
UDI Unique device identifier
QTY Quantity
REF Catalogue Number
Manufacturer
CONTROL + Positive control
Temperature Limit
Caution
Consult instructions for use
Use-by date

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