Mimix™ OncoSpan gDNA Reference Standard

IVD

For In Vitro Diagnostic Use

REF HD827-IVD Mimix™ OncoSpan gDNA Reference Standard

Intended Use

The Mimix™ OncoSpan gDNA reference standard is a commutable control material comprising a mixture of genomic DNA (gDNA) derived from human cancer cell lines, containing more than 380 variants across 152 cancer genes. It is intended for qualitative and/or quantitative monitoring of next-generation sequencing (NGS) and droplet digital polymerase chain reaction (ddPCR) assays designed to detect somatic mutations in human gDNA samples for *in vitro* diagnostic use.

This control can be used to monitor NGS and 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 analyse 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 analysed real time[3].

The Mimix™ OncoSpan gDNA Reference Standard enables direct analysis of patient-derived DNA samples against a wide range of 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 analyse patient-derived samples.

OncoSpan gDNA is a gDNA reference standard derived from well-characterized cell lines, mimicking patient-derived gDNA samples. It contains more than 380 variants across 152 key cancer genes, with variant allele frequencies ranging from 1% to 92.5% with 52 variants present at ≤ 20% allelic frequency for limit of detection determination of your assay. This diversity allows for comprehensive assessment of assay performance and quantitative accuracy.

The Mimix™ OncoSpan gDNA Reference Standard is a multiplex standard covering the mutations on the following genes: ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ATR, ATRX, AXL, BARD1, BCL6, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BTK, BUB1B, CARD11, CCND1, CCND3, CCNE1, CD79B, CDh2, CDK12, CDK4, CEP57, CFH, CREBBP, CSF1R, CTNNB1, DDR2, DIS3L2, DNMT3A, EGFR, EML4, EP300, EPCAM, ERBB2, ERBB3, ERCC1, ERCC2, ERCC4, ERCC5, ERG, ETS1, ETV4, EWSR1, EXT1, FANCA, FANCD2, FANCE, FANCG, FANCI, FANCM, FBXW7, FGF10, FGF2, FGF3, FGF6, FGFR1, FGFR3, FLCN, FLI1, FLT1, FLT3, FZR1, GATA2, GATA3, GEN1, GNA11, GNAS, HNF1A, HRAS, IDh2, IDh4, JAK1, JAK2, JAK3, KDR, KIT, KRAS, LDLR, MAGI1, MAP2K1, MAP2K2, MAX, MDM4, MED12, MET, MLh2, MLLT3, MMAB, MRE11, MSh4, MSH6, MTOR, NBN, NF1, NFE2L2, NOTCh1, NOTCh4, NOTCh6, NRAS, NRG1, NTRK1, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3CD, PIK3CG, PIK3R1, PMS2, PPARG, PPP2R2A, PRKAR1A, PROC, PTCh2, PTPN11, RAD51B, RAD54L, RAF1, RB1, RBM45, RECQL4, RET, RHBDF2, ROS1, RPS6KB1, SDHB, SF3B1, SF3B2, SLTM, SLX4, SMARCB1, SMO, SMOX, STK11, TERT, TET2, TFRC, TP53, TP53BP1, TSC1, TSC2, WRN, XPA, XPC, ZNF395.

Principles of Operation

The Mimix™ OncoSpan gDNA Reference Standard contains genomic DNA stored in Tris-EDTA, isolated from well-characterised cell lines to mimic patient samples. No synthetic DNA is incorporated into this product. The gDNA is intended to be integrated into the customer's workflow for DNA extraction. Every batch of OncoSpan DNA has 25 variants confirmed by ddPCR (Table 1), in addition to being fully exome sequenced. This provides an accurate and reliable set for comparison to any NGS assay's performance.

In addition to ddPCR and NGS, further quality control of this product was performed via spectrophotometry.

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

Chromosome	Gene	Variant	Expected AF	Acceptance Range
chr2 (29416025)	ALK	N/A (Ins)	10.0%	9.00 – 11.00%
chr5 (112175770)	APC	p.T1493T	35.0%	31.50 – 38.50%
chr7 (140453136)	BRAF	p.V600E	10.5%	8.95 - 11.05%
chr13 (32913558)	BRCA2	p.K1691fs*15	32.5%	29.25 – 35.75%
chr3 (41266101)	CTNNB1	p.S33Y	32.5%	29.25 – 35.75%
chr3 (41266133)	CTNNB1	p.S45del	10.0%	9.00 – 11.00%
chr7 (55241707)	EGFR	p.G719S	24.5%	22.05 – 26.95%
chr7 (55249071)	EGFR	p.T790M	1.0%	0.70 – 1.30%
chr7 (55259515)	EGFR	p.L858R	3.0%	2.10 – 3.90%
chr7 (55242464)	EGFR	p.E746_A750 delELREA	2.0%	14.0 – 2.60%
chr7 (55249063)	EGFR	p.Q787Q	15.0%	13.50 – 16.50%
chr4 (153244155)	FBXW7	p.S668fs*39	32.5%	29.25 – 35.75%
chr13 (28578214)	FLT3	p.P986fs*>8	10.0%	9.00 – 11.00%
chr4 (55599321)	KIT	p.D816V	10.0%	9.00 – 11.00%
chr4 (55602765)	KIT	p.L862L	7.5%	6.00 – 9.00%
chr12 (25398281)	KRAS	p.G13D	15.0%	13.50 – 16.50%
chr12 (25398284)	KRAS	p.G12D	6.0%	4.80 – 7.20%
chr7 (116436022)	MET	p.A1357A	7.0%	5.60 – 8.40%
chr7 (116339847)	MET	p.L238fs*25	7.0%	5.60 – 8.40%
chr9 (139409754)	NOTCh1	p.P668S	30.0%	27.00 – 33.00%
chr1 (115256530)	NRAS	p.Q61K	12.5%	11.25 – 13.75%
chr3 (178936091)	PIK3CA	p.E545K	9.0%	7.20 – 10.80%
chr3 (178952085)	PIK3CA	p.H1047R	17.5%	15.75 – 19.25%
chr10 (43613843)	RET	p.L769L	60.0%	54.00 – 66.00%
chr17 (7579472)	TP53	p.P72R	92.5%	83.25 – 100.00%

Material Provided: Reagents

Mimix™ OncoSpan gDNA Reference Standard

- Catalogue/Model number: HD827-IVD
  One vial, 1x 20µL
  Material provided in Tris-EDTA (10 mM Tris-HCl, 1 mM EDTA), pH 8.0.

⚠ Precautions and Warning

- Although this product is not classified as dangerous, handle all materials as potentially biohazardous, following standard laboratory safety procedures.
- Dispose of all waste materials in accordance with local regulations.
- Inspect the vial for any signs of damage or leakage.

Storage and Handling

- Store unopened at 2 to 8 °C.
- Do not use this product beyond the expiration date printed on the product label.

Interpretation of Results

Variant detection results for the Mimix™ OncoSpan gDNA 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 gDNA provided can be used to compare each subsequent run.

Quality Control and Performance Characteristics

Variant detection when using the Mimix™ OncoSpan gDNA 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 gDNA 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 were obtained using the validated equipment and workflow as specified for each quality control test. Spectrophotometry and ddPCR data are provided in Figures 1 and 2 respectively.

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

Test name	Test purpose	Acceptance criteria
Spectrophotometry	Measure quantity and purity of FFPE DNA	Concentration: 48-52 ng/µL 260nm/280nm ratio: 1.75-2.00
ddPCR	Measure stability of AF	Within the accepted target range. Refer to Table 1.

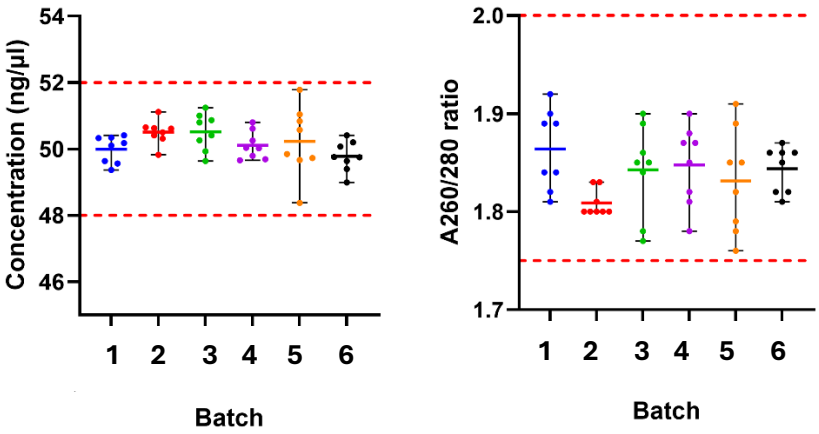

Figure 1. A. Concentration of OncoSpan gDNA measured by spectrophotometry. B. A260/280 ratio of OncoSpan gDNA measured by spectrophotometry. The graphs display upper and lower limits of the acceptance range (red dotted lines in A and B).

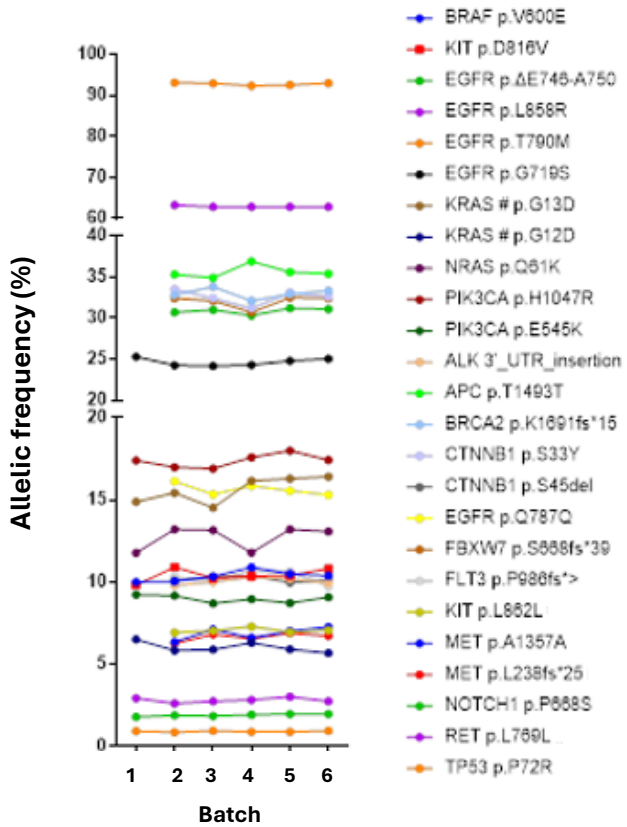

Figure 2. Changes in mutation allelic frequency over time. The observed allelic frequencies are stable over time.

Limitations

- The Mimix™ OncoSpan gDNA 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
Tel: 800-235-9880 (Option 2) Or 303-604-9499 (Option 2)
Fax: 1-800-292-6088 Or 303-604-9680
Email: technical@horizondiscovery.com

Customer support
Tel: 800-235-9880 (Option 1) Or 303-604-9499 (Option 1)
Fax: 1-800-292-6088 Or 303-604-9680
Email: orders@horizondiscovery.com

For a high-confidence variant list go to https://horizondiscovery.com/reference-standards/products/oncospan-gdna-reference-standard-ivd

Batch-specific whole exome sequencing data (pro ' led upon purchase as an online link).

References

1 Vinkšel, M., Writzl, K., Maver, A. *et al*. Improving diagnostics of rare genetic diseases with NGS approaches. *J Community Genet* 12, 247–256 (2021). https://doi.org/10.1007/s12687-020-00500-5

2 Boycott K, Hartley T, Adam S, et al. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet*. 2015;52(7):431-437. doi:10.1136/jmedgenet-2015-103144

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

Glossary

IVD In Vitro Diagnostic Medical Device

REF Catalogue Number

Legal Manufacturer

Temperature Limit

Caution

Non-sterile

Consult Instructions for Use

Use-by date

UDI Unique device identifier

QTY Quantity

CONTROL + Positive control

Trademark and Patent information

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