

Multiplex I cfDNA Reference Standard Set in a Synthetic Matrix

Product Handling:

Horizon Discovery recommends quantifying the material using preferred laboratory method and instrumentation prior to use.

Origin of Material:

- Horizon Discovery uses a proprietary genome engineering platform to precisely engineer mutations within human cancer cell lines.
- The cell line DNA is fragmented to an average fragment length of 170 bp which resembles cfDNA extracted from plasma.
- Additional information can be found on Product Specification Sheet (6245 PSS-02) on the website for this catalogue number.
- Fragmented DNA is spiked into a synthetic matrix.

Allelic Frequency Verification Data:

- Data is provided in Table 1-4. These values represent only the QC assays performed by Horizon using an input of **50 ng** of cfDNA extracted from synthetic matrix, and are an average of 4 replicates. Wild type and mutant copy numbers are rounded up to the nearest whole number.
- Whole-exome sequencing data from the wild type cell lines, RKO and SW48, are available upon registration at: www.horizondiscovery.com/exomedata

Table 1: 5% Multiplex I cfDNA Reference Standard in a Synthetic Matrix (Part No.: HD812)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Copies	Mutant Copies	Total Copies
EGFR	L858R	78.0	5.0	11640	596	12236
EGFR	delE746-A750	110/95	5.0	6780	416	7196
EGFR	T790M	117.0	5.0	5920	312	6232
EGFR	V769-D770insASV	122/131	5.0	5694	260	5954
KRAS	G12D	80.0	6.3	9440	610	10050
NRAS	Q61K	83.0	6.3	9900	660	10560
NRAS	A59T	66.0	6.3	11320	774	12094
PIK3CA	E545K	81.0	6.3	10020	648	10668

Table 2: 1% Multiplex I cfDNA Reference Standard in a Synthetic Matrix (Part No.: HD813)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Copies	Mutant Copies	Total Copies
EGFR	L858R	78.0	1.0	13940	138	14078
EGFR	delE746-A750	110/95	1.0	8360	110	8470
EGFR	T790M	117.0	1.0	7060	74	7134
EGFR	V769-D770insASV	122/131	1.0	6740	66	6806
KRAS	G12D	80.0	1.3	10840	150	10990
NRAS	Q61K	83.0	1.3	11520	156	11676
NRAS	A59T	66.0	1.3	13820	200	14020
PIK3CA	E545K	81.0	1.3	11440	148	11588

Table 3: 0.1% Multiplex I cfDNA Reference Standard in a Synthetic Matrix (Part No.: HD814)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Copies	Mutant Copies	Total Copies
EGFR	L858R	78.0	0.10	13200	16	13216
EGFR	delE746-A750*	110/95	0.10	7680	9	7689
EGFR	T790M*	117.0	0.10	6800	11	6811
EGFR	V769-D770insASV	122/131	0.10	6660	5	6665
KRAS	G12D*	80.0	0.13	10300	17	10317
NRAS	Q61K	83.0	0.13	11800	16	11816
NRAS	A59T*	66.0	0.13	13140	22	13162
PIK3CA	E545K*	81.0	0.13	11040	15	11055

Table 4: 100% Multiplex I wt cfDNA Reference Standard in a Synthetic Matrix (Part No.: HD815)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Copies	Mutant Copies	Total Copies
EGFR	L858R	78.0	0.00	13340	1	13341
EGFR	delE746-A750	110/95	0.00	7820	0	7820
EGFR	T790M	117.0	0.00	6940	3	6943
EGFR	V769-D770insASV	122/131	0.00	6820	0	6820
KRAS	G12D	80.0	0.00	10260	1	10261
NRAS	Q61K	83.0	0.00	10980	0	10980
NRAS	A59T	66.0	0.00	13260	2	13262
PIK3CA	E545K	81.0	0.00	11080	1	11081

Table 1-4: Allelic frequency and copy number data collected by Droplet Digital™ PCR (ddPCR). Other endogenous variants are present, however only the data presented is tested within each batch. *Not verified for expected AF, due to assay background, however variant was detected by ddPCR. *Not verified for expected AF, due to QC assay used demonstrated probe competition with NRAS Q61K, however variant was detected by ddPCR.