

Product Insert

Set Cat No: HD780

Batch: 30458

Template: 6135 USR-01 (V-03)

Multiplex HD780 cfDNA Reference Standard Set

Product Handling:

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

Origin of Material:

- Horizon Diagnostics 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 160 bp which resembles cfDNA extracted from plasma.
- Additional information can be found on Product Specification Sheet (6135 PSS) on the website for this catalogue number.

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 100 ng and are an average of 4 replicates (WT Conc (copies/μl) and Mutant Conc (copies/μl) values rounded to whole numbers, with the exception of 100% Multiplex I wt Mutant Conc (copies/μl) which is reported to 1 decimal place).
- 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 (Part No.: HD777)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Conc (copies/μl)	Mutant Conc (copies/μl)	Total Conc (copies/μl)
EGFR	T790M	117.0	5.0	2120	109	2229
EGFR	delE746-A750	110/95	5.0	2388	146	2534
EGFR	L858R	78.0	5.0	4092	203	4295
EGFR	V769-D770insASV	122/131	5.0	2108	97	2205
NRAS	Q61K	83.0	6.3	3316	224	3540
NRAS	A59T	66.0	6.3	3628	262	3890
PIK3CA	E545K	81.0	6.3	3340	210	3550
KRAS	G12D	80.0	6.3	3152	197	3349

Table 2: 1% Multiplex I cfDNA Reference Standard (Part No.: HD778)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Conc (copies/μl)	Mutant Conc (copies/μl)	Total Conc (copies/μl)
EGFR	T790M	117.0	1.0	2256	24	2280
EGFR	delE746-A750	110/95	1.0	2468	30	2498
EGFR	L858R	78.0	1.0	4384	40	4424
EGFR	V769-D770insASV	122/131	1.0	2204	19	2223
NRAS	Q61K	83.0	1.3	3568	46	3614
NRAS	A59T	66.0	1.3	4360	56	4416
PIK3CA	E545K	81.0	1.3	3576	44	3620
KRAS	G12D	80.0	1.3	3308	36	3344

Table 3: 0.1% Multiplex I cfDNA Reference Standard (Part No.: HD779)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Conc (copies/μl)	Mutant Conc (copies/μl)	Total Conc (copies/μl)
EGFR	T790M	117.0	0.10	2228	3	2231
EGFR	delE746-A750	110/95	0.10	2428	2	2430
EGFR	L858R	78.0	0.10	4188	4	4192
EGFR	V769-D770insASV	122/131	0.10	2224	2	2226
NRAS	Q61K	83.0	0.13	3536	5	3541
NRAS	A59T	66.0	0.13	4012	5	4017
PIK3CA	E545K	81.0	0.13	3528	5	3533
KRAS	G12D	80.0	0.13	3268	5	3273

Table 4: 100% Multiplex I wt cfDNA Reference Standard (Part No.:HD776)

Gene	Variant	Amplicon size (bp)	Expected AF (%)	WT Conc (copies/μl)	Mutant Conc (copies/μl)	Total Conc (copies/μl)
EGFR	T790M	117.0	0.00	2248	0.7	2249
EGFR	delE746-A750	110/95	0.00	2572	0.0	2572
EGFR	L858R	78.0	0.00	4408	0.1	4408
EGFR	V769-D770insASV	122/131	0.00	2216	0.0	2216
NRAS	Q61K	83.0	0.00	3492	0.1	3492
NRAS	A59T	66.0	0.00	4184	1.0	4185
PIK3CA	E545K	81.0	0.00	3628	0.6	3629
KRAS	G12D	80.0	0.00	3380	1.1	3381

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.**