

Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description: Seraseq™ ctDNA Complete™ Reference Material AF 1%

Material No: 0710-0671

Batch No: 10346907

Material Description: Mixture of human genomic DNA from the reference cell line, GM24385, and synthetic DNA constructs

Date of Manufacture: 16May2018

Expiration Date: 16May2020

Concentration (Qubit dsDNA BR Assay): Nominal value: 25 ng/mL; Average measured value after extraction using Qiagen QIAamp Circulating Nucleic Acid Kit: 31.4 ng/mL

Volume: 5 mL

Storage: 4 °C

Gene ID	COSMIC Identifier	Amino Acid Change	Average AF%
AKT1	COSM33765	p.E17K	1.06
BRAF	COSM476	p.V600E	1.02
EGFR	COSM6224	p.L858R	1.07
EGFR	COSM6240	p.T790M	0.99
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.94
KIT	COSM1314	p.D816V	1.07
KRAS	COSM521	p.G12D	1.09
NCOA4/RET	NA	Translocation	1.03
NRAS	COSM584	p.Q61R	1.09
PIK3CA	COSM775	p.H1047R	1.09
PIK3CA	COSM12464	p.N1068fs*4	1.09
EML4-ALK	NA	Translocation	0.97
ALK	COSM144250	p.G1202R	1.03
ALK	COSM28055	p.F1174L	1.03
BRCA1	COSM1383519	p.K654fs*47	0.99
BRCA2	COSM1738242	p.R2645fs*3	0.94
EGFR	COSM12370	p.L747_P753>S	1.15
EGFR	COSM6256	p.S752_I759delSPKANKEI	1.06
EGFR	COSM6223	p.E746_A750delELREA	1.18
KRAS	COSM516	p.G12C	1.04
CD74/ROS1	NA	Translocation	1.02
KRAS	COSM554	p.Q61H	0.97
			Average AF%
			1.04

Digital PCR testing using BioRad QX200™ Droplet Digital™ PCR System:

Gene ID	Average CNV in ctDNA ¹	Average Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell ²
ERBB2	2.87	0.87	44
MET	2.68	0.68	34
MYC	3.07	1.07	54

NA = not applicable

¹Compare to a normal CNV of 2.00.

²Calculated based on the ctDNA fraction of 2%.

Next Generation Sequencing testing using Archer® Reveal ctDNA™ 28 Kit run on an Illumina® MiSeq™ using v2 (2x150 bp) PE chemistry reagents^{1,2}:

Gene ID	COSMIC Identifier	Amino Acid Change	AF%
AKT1	COSM33765	p.E17K	0.96
BRAF	COSM476	p.V600E	1.08
EGFR	COSM6224	p.L858R	1.01
EGFR	COSM6240	p.T790M	1.56
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.64
KIT	COSM1314	p.D816V	1.36
KRAS	COSM521	p.G12D	1.02
NCOA4/RET	NA	Translocation	NA
NRAS	COSM584	p.Q61R	1.42
PIK3CA	COSM775	p.H1047R	1.09
PIK3CA	COSM12464	p.N1068fs*4	0.90
EML4-ALK	NA	Translocation	NA
ALK	COSM144250	p.G1202R	1.01
ALK	COSM28055	p.F1174L	0.99
BRCA1	COSM1383519	p.K654fs*47	NA
BRCA2	COSM1738242	p.R2645fs*3	NA
EGFR	COSM12370	p.L747_P753>S	0.87
EGFR	COSM6256	p.S752_I759delSPKANKEI	1.00
EGFR	COSM6223	p.E746_A750delELREA	1.12
KRAS	COSM516	p.G12C	1.11
CD74/ROS1	NA	Translocation	NA
KRAS	COSM554	p.Q61H	0.90
Average AF%			1.06

Gene ID	CNV in ctDNA ³	Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell ⁴
ERBB2	2.90	0.90	45
MET	2.92	0.92	46
MYC	NA	NA	NA

NA = not applicable; AF% and CNV marked NA were not targeted by the panel.

¹NGS was performed as an orthogonal verification step. Parameters used:

DNA input = 50 ng

of samples / flow cell = 3-4

of total reads / sample = 4-5M

Average read depth = 5000-10000X

On-target reads = ~94%

Q30 score = ~95%

Analysis = Archer Analysis Suite v5.1.7 (with error correction set to "ON")

²Please see the poster from NIST for more information about assay sensitivity:

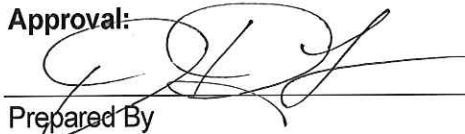
<https://digital.seracare.com/multilab-assessment-reference-materials-ctdna-poster2018>

³Compare to a normal CNV of 2.00.

⁴Calculated based on the ctDNA fraction of 2%.

Note: The MET gene is amplified using two synthetic constructs with a small region of overlap between the constructs (see package insert for genomic coordinates). Assays which target this region of overlap may report higher amplification levels.

Approval:



Prepared By

03/21/19

Date