

Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description: Seraseq™ ctDNA Complete Reference Material AF 5%

Material No: 0710-0669

Batch No: 10346905

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: 28.3 ng/mL

Volume: 5 mL

Storage: 4 °C

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

Gene ID	COSMIC Identifier	Amino Acid Change	Average AF%
AKT1	COSM33765	p.E17K	5.22
BRAF	COSM476	p.V600E	5.26
EGFR	COSM6224	p.L858R	5.3
EGFR	COSM6240	p.T790M	5.03
ERBB2	COSM682/20959	p.A775_G776insYVMA	5.12
KIT	COSM1314	p.D816V	5.18
KRAS	COSM521	p.G12D	5.35
NCOA4/RET	NA	Translocation	5.07
NRAS	COSM584	p.Q61R	5.47
PIK3CA	COSM775	p.H1047R	4.98
PIK3CA	COSM12464	p.N1068fs*4	4.98
EML4-ALK	NA	Translocation	4.58
ALK	COSM144250	p.G1202R	4.88
ALK	COSM28055	p.F1174L	4.88
BRCA1	COSM1383519	p.K654fs*47	4.79
BRCA2	COSM1738242	p.R2645fs*3	4.61
EGFR	COSM12370	p.L747_P753>S	6.02
EGFR	COSM6256	p.S752_I759delSPKANKEI	5.14
EGFR	COSM6223	p.E746_A750delELREA	5.72
KRAS	COSM516	p.G12C	5.28
CD74/ROS1	NA	Translocation	4.88
KRAS	COSM554	p.Q61H	4.89
Average AF%			5.12

Gene ID	Average CNV in ctDNA ¹	Average Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell ²
ERBB2	7.67	5.67	57
MET	6.11	4.11	41
MYC	6.27	4.27	43

NA = not applicable

¹Compare to a normal CNV of 2.00.

²Calculated based on the ctDNA fraction of 10%.

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	4.57
BRAF	COSM476	p.V600E	5.44
EGFR	COSM6224	p.L858R	4.54
EGFR	COSM6240	p.T790M	5.05
ERBB2	COSM682/20959	p.A775_G776insYVMA	3.44
KIT	COSM1314	p.D816V	5.59
KRAS	COSM521	p.G12D	6.11
NCOA4/RET	NA	Translocation	NA
NRAS	COSM584	p.Q61R	5.79
PIK3CA	COSM775	p.H1047R	4.99
PIK3CA	COSM12464	p.N1068fs*4	4.08
EML4-ALK	NA	Translocation	NA
ALK	COSM144250	p.G1202R	4.21
ALK	COSM28055	p.F1174L	5.08
BRCA1	COSM1383519	p.K654fs*47	NA
BRCA2	COSM1738242	p.R2645fs*3	NA
EGFR	COSM12370	p.L747_P753>S	6.48
EGFR	COSM6256	p.S752_I759delSPKANKEI	5.64
EGFR	COSM6223	p.E746_A750delELREA	5.81
KRAS	COSM516	p.G12C	4.58
CD74/ROS1	NA	Translocation	NA
KRAS	COSM554	p.Q61H	4.30
Average AF%			5.04

Gene ID	CNV in ctDNA ³	Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell ⁴
ERBB2	6.40	4.40	44
MET	8.50	6.50	65
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 10%.

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