

# Technical Product Report

*For Research Use Only; Not for use in Diagnostic Procedures*

Product Description: Seraseq™ ctDNA Complete™ Reference Material AF 0.1%

Material No: 0710-0673

Batch No: 10346909

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

Date of Manufacture: 17May2018

Expiration Date: 17May2020

Concentration (Qubit dsDNA BR Assay): Nominal value: 25 ng/mL; Average measured value after extraction using Qiagen QIAamp Circulating Nucleic Acid Kit: 28.4 ng/mL

Volume: 5 mL

Storage: 4 °C

Gene ID	COSMIC Identifier	Amino Acid Change	Average AF%
AKT1	COSM33765	p.E17K	0.118
BRAF	COSM476	p.V600E	0.104
EGFR	COSM6224	p.L858R	0.106
EGFR	COSM6240	p.T790M	0.116
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.089
KIT	COSM1314	p.D816V	0.100
KRAS	COSM521	p.G12D	0.115
NCOA4/RET	NA	Translocation	0.094
NRAS	COSM584	p.Q61R	0.114
PIK3CA	COSM775	p.H1047R	0.106
PIK3CA	COSM12464	p.N1068fs*4	0.106
EML4-ALK	NA	Translocation	0.083
ALK	COSM144250	p.G1202R	0.100
ALK	COSM28055	p.F1174L	0.100
BRCA1	COSM1383519	p.K654fs*47	0.103
BRCA2	COSM1738242	p.R2645fs*3	0.106
EGFR	COSM12370	p.L747_P753>S	0.113
EGFR	COSM6256	p.S752_I759delSPKANKEI	0.109
EGFR	COSM6223	p.E746_A750delELREA	0.120
KRAS	COSM516	p.G12C	0.089
CD74/ROS1	NA	Translocation	0.107
KRAS	COSM554	p.Q61H	0.106
<b>Average AF%</b>			<b>0.105</b>

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

Gene ID	Average CNV in ctDNA <sup>1</sup>	Average Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell <sup>2</sup>
ERBB2	2.18	0.18	90
MET	2.05	0.05	25
MYC	2.09	0.09	45

NA = not applicable

<sup>1</sup>Compare to a normal CNV of 2.00.

<sup>2</sup>Calculated based on the ctDNA fraction of 0.2%. Values are within the noise of the copy number changes and just above normal diploid copy number resulting in variability.

NGS was performed as an orthogonal verification step. Results confirm all variants were detected in the expected range given the parameters used for testing, with the exception of PIK3CA COSM12464 due to the poor quality of reads mapped to this variant; the corresponding dPCR data was 0.106%. Numerical data is not included for this product as assay sensitivity<sup>1</sup> is not sufficient for confidence in measured AF values.

Next Generation Sequencing testing using Archer<sup>®</sup> Reveal ctDNA<sup>™</sup> 28 Kit run on an Illumina<sup>®</sup> MiSeq<sup>™</sup> using v2 (2x150 bp) PE chemistry reagents:

**NGS Parameters:**

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")

<sup>1</sup>Please see the poster from NIST for more information about assay sensitivity:  
<https://digital.seracare.com/multilab-assessment-reference-materials-ctdna-poster2018>

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:

A handwritten signature in black ink, appearing to be "R. A. ...".

Prepared By

03/21/19

Date