

Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description: Seraseq™ ctDNA Complete™ Reference Material AF 0.5%

Material No: 0710-0672

Batch No: 10346908

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	0.51
BRAF	COSM476	p.V600E	0.48
EGFR	COSM6224	p.L858R	0.54
EGFR	COSM6240	p.T790M	0.46
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.42
KIT	COSM1314	p.D816V	0.53
KRAS	COSM521	p.G12D	0.56
NCOA4/RET	NA	Translocation	0.56
NRAS	COSM584	p.Q61R	0.54
PIK3CA	COSM775	p.H1047R	0.55
PIK3CA	COSM12464	p.N1068fs*4	0.55
EML4-ALK	NA	Translocation	0.49
ALK	COSM144250	p.G1202R	0.56
ALK	COSM28055	p.F1174L	0.56
BRCA1	COSM1383519	p.K654fs*47	0.46
BRCA2	COSM1738242	p.R2645fs*3	0.50
EGFR	COSM12370	p.L747_P753>S	0.59
EGFR	COSM6256	p.S752_I759delSPKANKEI	0.50
EGFR	COSM6223	p.E746_A750delELREA	0.64
KRAS	COSM516	p.G12C	0.52
CD74/ROS1	NA	Translocation	0.53
KRAS	COSM554	p.Q61H	0.56
			Average AF%
			0.53

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	2.56	0.56	56
MET	2.41	0.41	41
MYC	2.37	0.37	37

NA = not applicable

¹Compare to a normal CNV of 2.00.

²Calculated based on the ctDNA fraction of 1%.

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	0.63
BRAF	COSM476	p.V600E	0.62
EGFR	COSM6224	p.L858R	0.55
EGFR	COSM6240	p.T790M	0.68
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.27
KIT	COSM1314	p.D816V	0.73
KRAS	COSM521	p.G12D	0.62
NCOA4/RET	NA	Translocation	NA
NRAS	COSM584	p.Q61R	0.98
PIK3CA	COSM775	p.H1047R	0.66
PIK3CA	COSM12464	p.N1068fs*4	0.58
EML4-ALK	NA	Translocation	NA
ALK	COSM144250	p.G1202R	0.35
ALK	COSM28055	p.F1174L	0.45
BRCA1	COSM1383519	p.K654fs*47	NA
BRCA2	COSM1738242	p.R2645fs*3	NA
EGFR	COSM12370	p.L747_P753>S	0.43
EGFR	COSM6256	p.S752_I759delSPKANKEI	0.35
EGFR	COSM6223	p.E746_A750delELREA	0.42
KRAS	COSM516	p.G12C	0.62
CD74/ROS1	NA	Translocation	NA
KRAS	COSM554	p.Q61H	0.58
Average AF%			0.56

Gene ID	CNV in ctDNA ³	Additional Copies (per cell) in ctDNA	Approx. CNV in Tumor Cell ⁴
ERBB2	2.60	0.60	60
MET	2.64	0.64	64
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 1%.

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