Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description:	Seraseq® ctDNA Complete Reference Material WT		
Material Number:	0710-0674	Batch Number:	10557979
Material Description:	A ctDNA-like mixture of human genomic DNA from the reference cell line, GM24385		
Concentration (Qubit dsDNA BR Assay):	Nominal value: 25 ng/mL; Average measured value after extraction using Qiagen QIAamp Circulating Nucleic Acid Kit: 27.8 ng/mL		
Fill Volume:	5.0 mL		
Date of Manufacture:	13-MAY-2021	Expiration Date:	13-MAY-2024
Storage:	2-8°C		

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

Gene ID	COSMIC Identifier	Amino Acid Change	Average AF%[1]
AKT1	COSM33765	p.E17K	0.005
BRAF	COSM476	p.V600E	0.000
EGFR	COSM6224	p.L858R	0.001
EGFR	COSM6240	p.T790M	0.009
ERBB2	COSM20959	p.A775_G776insYVMA	0.007
KIT	COSM1314	p.D816V	0.002
KRAS	COSM521	p.G12D	0.012
NCOA4/RET	NA	Translocation	0.000
NRAS	COSM584	p.Q61R	0.006
PIK3CA	COSM775	p.H1047R	0.006
PIK3CA	COSM12464[2]	p.N1068fs*4	0.006
EML4-ALK	NA	Translocation	0.001
ALK	COSM144250	p.G1202R	0.000
ALK	COSM28055	p.F1174L	0.000
BRCA1	COSM1383519	p.K654fs*47	0.001
BRCA2	COSM1738242	p.R2645fs*3	0.000
EGFR	COSM12370	p.L747_P753>S	0.000
EGFR	COSM6256	p.S752_I759delSPKANKEI	0.001
EGFR	COSM6223	p.E746_A750delELREA	0.008
KRAS	COSM516	p.G12C	0.001
CD74/ROS1	NA	Translocation	0.000
KRAS	COSM554	p.Q61H	0.004

NA = not applicable

[1]Variant allele frequencies > 0.00% for this wild-type negative control are within the expected range for stochastic positive dPCR reactions.

[2]As of June 2019, this mutation is no longer listed in the COSMIC database.

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Next Generation Sequencing testing using Archer® Reveal ctDNA™ 28 Kit run on an Illumina® MiSeq™ using v2 (2x150 bp) PE chemistry reagents[1]	NGS was performed as an orthogonal verification step. Results confirm no variants were detected above 0.1%, except for EGFR T790M variant detected at 0.11% in the WT sample; the corresponding dPCR data was 0.009%. We observed similar background in other Seraseq ctDNA Complete™ reference materials using this NGS assay, which were not observed in dPCR QC testing. Thus, we associate the allele frequency for the T790M variant with the background from the assay using the conditions listed below.

NGS Parameters:
DNA input = 50 ng
# of samples / flow cell = 1
# of total reads / sample = 5.6M
Average read depth = 4490X
On-target reads = 94.6%
Q30 score = 94.1%
Analysis = Archer Analysis Suite v6.2.2 (default settings except for: Variant Downstream ROI Size of 150 bp and Read Depth NOrmalization of 10000000)

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

NOTE: Copy numbers of ERBB2 and MET were assayed by dPCR and found to be normal. They were not assayed by NGS as the wild-type sample is used as a normal control for determining copy number of genes in other samples.

Approval:

Prepared By	27 MAY 2021 Date