Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description:	Seraseq® ctDNA Mutation Mix v2 WT		
Material Number:	0710-0144	Batch Number:	10613512
Material Description:	ctDNA-like human genomic DNA from the reference cell line, GM24385.		
Concentration (Qubit dsDNA BR Assay):	16.1 ng/µL		
Volume:	25 µL		
Date of Manufacture:	18 MAY 2022	Expiration Date:	18 MAY 2024
Storage:	-20°C		

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

Gene ID	COSMIC Identifier	AF%[1]
AKT1	COSM33765	0.000
APC	COSM13127	0.000
APC	COSM18561	0.000
ATM	COSM21924	0.000
BRAF	COSM476	0.000
CTNNB1	COSM5664	0.000
EGFR	COSM6224	0.000
EGFR	COSM12378	0.000
EGFR	COSM6225	0.000
EGFR	COSM6240	0.000
ERBB2	COSM20959	0.000
FGFR3	COSM715	0.000
FLT3	COSM783	0.000
FOXL2	COSM33661	0.000
GNA11	COSM52969	0.000
GNAQ	COSM28758	0.000
GNAS	COSM27887	0.000
IDH1	COSM28747	0.000
JAK2	COSM12600	0.000
KIT	COSM1314	0.000
KRAS	COSM521	0.000
MPL	COSM18918	0.000
NCOA4-RET	N/A	0.000
NPM1	COSM17559	0.000
NRAS	COSM584	0.000
PDGFRA	COSM736	0.000
PDGFRA	COSM28053	0.000
PIK3CA	COSM763	0.000
PIK3CA	COSM12464 [3]	0.000
PIK3CA	COSM775	0.000
PTEN	COSM4986	0.000
PTEN	COSM5809	0.000
RET	COSM965	0.000
SMAD4	COSM14105	0.000
TP53	COSM10648	0.000
TP53	COSM10660	0.000
TP53	COSM10662	0.000
TP53	COSM6530	0.000
TP53	COSM18610	Not Tested[2]
TPR-ALK	N/A	0.000

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

[2] NGS testing alone is used for QC of variant COSM18610.

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

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NGS was performed as an orthogonal verification step. Results confirm no variants were detected above 0.1%, except for the NRAS COSM584 variant detected at 0.14%, the TP53 COSM10648 variant detected at 0.12%, and the TP53 COSM10662 variant detected at 0.11% in the WT sample; the corresponding dPCR data were all 0.000%. Thus, we associate the allele frequency for these variants with the background from the assay using the conditions listed below.

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

NGS Parameters:
DNA input = 50 ng
# of samples / flow cell = 1
# of total reads / sample = 12.6 M
Average read depth = 7500X
On-target reads = ~94%
Q30 score = ~83%
Analysis = Archer Analysis Suite v6.2.7

Default analysis settings except for the following:

- NORMALIZATION_LEVEL is set to 10000000 (default is 3500000; cfDNA pipeline)
- VARIANT_DOWNSTREAM_ROI_SIZE is set to 150 (default=400; cfDNA pipeline)

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

Approval:

Prepared By — Date: 24 MAY 2022