Seraseq®

ctDNA Reference Material v4 WT, AF5%, AF0.5%, AF0.1% Package Insert

PLEASE NOTE:
THESE REAGENTS MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

NAME AND INTENDED USE

The Seraseq® ctDNA Reference Material v4 is intended for verifying the functionality of plasma cfDNA extraction workflows in combination with Next Generation Sequencing (NGS) assays that detect variants present in circulating tumor DNA (ctDNA) in the blood. The Seraseq ctDNA Reference Material v4 is intended as a reference material for translational and disease research testing and monitors cfDNA extraction, library preparation, sequencing, and variant allele detection under a given set of bioinformatics pipeline parameters. *For Research Use Only. Not for use in diagnostic procedures.*

SUMMARY

A well-designed quality control program can provide added confidence in the reliability of results obtained for unknown specimens. The use of independent reference products may provide valuable information concerning assay accuracy and bioinformatics pipeline analysis.

Seraseq ctDNA Reference Material v4 contains 93 mutations (not including those present in the GM24385 background) that are associated with ctDNA monitoring and are predominantly druggable mutations (see Table 2). The product is formulated to simulate ctDNA fragment sizes with a peak between 150-230 bp. Variant allele frequency (VAF) and copy gain, is confirmed by digital PCR. VAF and CNV status are also measured by NGS as reported in the batch-specific Technical Product Report.

REAGENTS

Table 1. Seraseq ctDNA Reference Material v4

Material No.	Product
0710-3992	Seraseq ctDNA Reference Material v4 AF0.1%
0710-3991	Seraseq ctDNA Reference Material v4 AF0.5%
0710-3990	Seraseq ctDNA Reference Material v4 AF5%
0710-3989	Seraseq ctDNA Reference Material v4 WT

Each Material No. is available as an individual product. Information in this Package Insert applies to all products.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq ctDNA Reference Material v4 and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq ctDNA Reference Material v4 is manufactured using genomic DNA extracted from the human cell line GM24385, which is a B-lymphocytic, male cell line from the Personal Genome Project offered by the NIGMS Human Genetic Cell Repository (https://catalog.coriell.org/1/NIGMS). Seraseq ctDNA Reference Material v4 is formulated in a dilution of SeraCare's SeraCon™ Matribase.

Safety Precautions

Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[1]. Do not pipette by mouth; do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping up with 0.5% sodium hypochlorite solution. Dispose of all specimens and materials used in testing as though they contain infectious agents.

Handling Precautions

Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS

Store Seraseq ctDNA Reference Material v4 frozen at -20 °C or colder. When stored in this fashion, Seraseq ctDNA Reference Material v4 will be stable through the expiration indicated on the vial label.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq ctDNA Reference Material v4 is a mixture of human genomic DNA and synthetic DNA constructs. It should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Provided

Seraseq ctDNA Reference Material v4 consists of a mixture of human genomic DNA and synthetic DNA constructs, fragmented to a size comparable to naturally occurring ctDNA with a peak fragment size of 150-230 bp. The DNA is introduced into SeraCare's SeraCon™ Matribase. Material must undergo extraction prior to input into NGS library preparation or most other DNA analysis workflows. QIAGEN QIAamp® Circulating Nucleic Acid Kit with carrier RNA (extraction) and Qubit dsDNA BR Assay Kit (quantification) were utilized to extract ctDNA from a 1 mL volume, in triplicate (each concentration), and yielded the concentration in ng/mL of DNA indicated on the label claim. Note: Yield may vary depending on extraction and quantification method used.

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Mix by vortexing to ensure a homogeneous solution and spin briefly. Seraseq ctDNA Reference Material v4 may be input into workflows in a manner consistent with plasma fractions prior to extraction. Seraseq ctDNA Reference Material v4 may be processed through library preparation and sequencing in parallel with test specimens. Refer to standard assay procedures in order to determine the amount of material to use.

It is recommended to extract the entire vial upon thawing. If smaller volume aliquots are to be extracted, it is recommended to aliquot into desired volumes and immediately freeze the aliquots that will not be extracted.

Quality Control

Although Seraseq ctDNA Reference Material v4 is designed to be present at the indicated target VAF, the product does not have assigned values for mutation frequencies. There are many reasons why assays may observe deviation from the representative data which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of Seraseq ctDNA Reference Material v4 with each assay system prior to its routine use.

INTERPRETATION OF RESULTS

Detection of the variants within Seraseq ctDNA Reference Material v4 may vary with different types of tests and different test kit lots. Since the reference material does not have an assigned value, the laboratory must establish a range for each lot of Seraseq ctDNA Reference Material v4. When results for the product are outside of the established acceptance range, it may indicate unsatisfactory test performance. Possible sources of error include: deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents or change in bioinformatics pipeline parameters.

LIMITATIONS OF THE PROCEDURE

Seraseq ctDNA Reference Material v4 MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

TEST PROCEDURES and INTERPRETATION OF RESULTS provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. Seraseq ctDNA Reference Material v4 is not a calibrator and should not be used for assay calibration. Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

EXPECTED RESULTS

Specific detection of variants and variant allele frequencies will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory should establish its own acceptance criteria. For example, the acceptable range for each variant might include all values within two standard deviations of the mean of 20 data points obtained in 20 runs[2]. Table 2 lists the variants in the product (verified by digital PCR).

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq ctDNA Reference Material v4 has been designed for use with NGS sequencing procedures for the purpose of evaluating assay performance. Seraseq ctDNA Reference Material v4 does not have assigned values. Procedures for implementing a quality assurance program and monitoring test performance on a routine basis must be established by each individual laboratory.

REFERENCES

1. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.
2. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline– Fourth Edition. CLSI document C24, 2016.

Table 2: List of Mutations Incorporated

Gene	Nucleotide Change	Transcript	Variant Type
AKT1	c.49G>A	NM_005163.2	SNV
AR	c.2623C>T	NM_000044.6	SNV
ATM	c.1058_1059del	NM_000051.4	Deletion
BRAF	c.1799T>A	NM_004333.6	SNV
BRCA1	c.1961del	NM_007294.4	Deletion
BRCA2	c.7934del	NM_000059.4	Deletion
CDKN2A	c.9_32dup	NM_000077.5	Insertion
CHEK1	c.676del	NM_001114122.3	Deletion
CHEK2	c.1116_1117delinsGT	NM_007194.4	INDEL
EGFR	c.2235_2249del	NM_005228.5	Deletion
EGFR	c.2303G>T	NM_005228.5	SNV
EGFR	c.2310_2311insGGT	NM_005228.5	Insertion
EGFR	c.2369C>T	NM_005228.5	SNV
EGFR	c.2389T>A	NM_005228.5	SNV
EGFR	c.2573T>G	NM_005228.5	SNV
ERBB2	c.2313_2324dup	NM_004448.4	Insertion
ESR1	c.1613A>G	NM_000125.4	SNV
FGFR3	c.746C>G	NM_000142.5	SNV
HRAS	c.182A>G	NM_005343.4	SNV
HRAS	c.37G>C	NM_005343.4	SNV
IDH1	c.394C>T	NM_005896.4	SNV
IDH2	c.419G>A	NM_002168.4	SNV
IDH2	c.515G>A	NM_002168.4	SNV
KIT	c.2361+67_2361+72delTTTTTT	NM_000222.3	Deletion

Gene	Nucleotide Change	Transcript	Variant Type
KIT	c.2447A>T	NM_000222.3	SNV
KRAS	c.183A>C	NM_004985.5	SNV
KRAS	c.34G>T	NM_004985.5	SNV
KRAS	c.35G>A	NM_004985.5	SNV
MAP2K1	c.370C>T	NM_002755.4	SNV
MAP4K3	c.246-2475_246-2470delTTTTTT	NM_003618.4	Deletion
MAP4K3	c.998-35_998-30delAAAAAA	NM_003618.4	Deletion
MET	c.3082+1del	NM_001127500.3	Deletion
MLH1	c.232_243delinsATGTAAGG	NM_000249.4	INDEL
MSH2	c.1662-12_1677del	NM_000251.3	Deletion
MSH2	c.942+20_942+29delAAAAAAAAAA	NM_000251.3	Deletion
MSH6	c.2056_2060delinsCTTCTACCTCAAAAA	NM_000179.3	INDEL
MTOR	c.6644C>A	NM_004958.4	SNV
NF1	c.3738_3747del	NM_001042492.3	Deletion
NRAS	c.182A>G	NM_002524.5	SNV
NTRK1	c.1783G>A	NM_002529.4	SNV
NTRK2	c.1915G>A	NM_006180.6	SNV
NTRK3	c.1867G>A	NM_001012338.3	SNV
PALB2	c.839del	NM_024675.4	Deletion
PDGFRA	c.2525A>T	NM_006206.6	SNV
PIK3CA	c.1633G>A	NM_006218.4	SNV
PIK3CA	c.3140A>G	NM_006218.4	SNV
PIK3CA	c.3203dup	NM_006218.4	Insertion
PIK3R1	c.1727_1729del	NM_181523.3	Deletion
PMS2	c.861_864del	NM_000535.7	Deletion
PTCH1	c.2307_2308delinsTT	NM_000264.5	INDEL
PTEN	c.800del	NM_000314.8	Deletion
PTEN	c.741dup	NM_000314.8	Insertion
RAD51C	c.242C>A	NM_058216.3	SNV
RAD51C	c.338dup	NM_058216.3	SNV
RAD51D	c.271A>T	NM_002878.4	SNV
RAD51D	c.392dup	NM_002878.4	SNV
RAF1	c.770C>T	NM_002880.4	SNV
RB1	c.751C>T	NM_000321.3	SNV
RET	c.2753T>C	NM_020975.6	SNV
SLC7A8	c.-231_-224delTTTTTTTT	NM_012244.4	Deletion
SMARCB1	c.118C>T	NM_003073.5	SNV
STK11	c.734+1G>T	NM_000455.5	SNV

Gene	Nucleotide Change	Transcript	Variant Type
TERT	c.-124C>T	NM_198253.3	SNV
TERT	c.-146C>T	NM_198253.3	SNV
TP53	c.723del	NM_000546.6	Deletion
TP53	c.743G>A	NM_000546.6	SNV
TP53	c.818G>A	NM_000546.6	SNV
TSC1	c.1263+1G>T	NM_000368.5	SNV
TSC2	c.2640-1G>A	NM_000548.5	SNV
VHL	c.481C>T	NM_000551.4	SNV
ZNF2	c.*1525_*1530delTTTTTT	NM_021088.4	Deletion
CD74::NRG1	Intron 6::Intron 5	NM_001025159.3::NM_013964.5	Translocation
CD74::ROS1	Intron 6::Intron 34	NM_001025159.3::NM_001378902.1	Translocation
COL1A1::PDGFB	Intron 25::Intron 1	NM_000088.3::NM_002608.3	Translocation
EML4::ALK	Intron 13::Intron 19	NM_019063.5::NM_004304.5	Translocation
ETV6::NTRK3	Intron 5::Intron 14	NM_001987.5::NM_002530.4	Translocation
FGFR2::BICC1	Intron 17::Intron 2	NM_000141.5::NM_001080512.3	Translocation
FGFR3::TACC3	Exon 18::Intron 7	NM_000142.5::NM_006342.3	Translocation
NCOA4::RET	Intron 7::Intron 11	NM_001145263.2::NM_020975.6	Translocation
PML::NTRK2	Intron 2::Intron 12	NM_002675.4::NM_006180.6	Translocation
TPM3::NTRK1	Intron 7::Intron 9	NM_153649.4::NM_002529.4	Translocation
AKT2	Amplification	19:40736224_40791252	CNV
CCND1	Amplification	NM_053056.3:11:69455924_69469242	CNV
CCNE1	Amplification	NM_001238.4:19:30302898_30315219	CNV
CDK4	Amplification	NM_000075.4:58141510_58146093	CNV
ERBB2	Amplification	NM_004448.4:17:37844347_37884911	CNV
FGF19	Amplification	NM_005117.3:11:69513006_69518790	CNV
FGF3	Amplification	NM_005247.4:11:69624736_69634184	CNV
FGF4	Amplification	NM_002007.4:69587797_69590109	CNV
FGFR1	Amplification	NM_023110.3:8:38268661_38326153	CNV
MET***	Amplification	NM_001127500.3:7:116312250_116438431	CNV
MYC	Amplification	NM_002467.6:8:128747680_128755197	CNV
MYCN	Amplification	NM_005378.6:2:16080672_16087126	CNV

* MET gene is covered using overlapping DNA constructs. The overlapping regions are expected to show higher copy number levels than the rest of the gene.

NOTE: Above list does not include variants present in the GM24385 background. Indels are defined as deletion/insertions less than 10 base pairs.