Seraseq®

ctDNA Mutation Mix 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 Mutation Mix v4 is intended for use with Next Generation Sequencing (NGS) assays that identify variants present in circulating tumor DNA (ctDNA) present in the blood. The Seraseq ctDNA Mutation Mix v4 is intended as a reference material for translational and disease research testing and monitors 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.

PRINCIPLES OF THE PROCEDURE

Seraseq ctDNA Mutation Mix v4 is ready-to-use in Next Generation Sequencing (NGS) assays in steps that follow DNA isolation; no further purification or DNA isolation is needed. The reference materials should follow the same workflow as unknown samples. The product contains DNA at a concentration of 10 ng/µL. The reference material is formulated in 1 mM Tris / 0.1 mM EDTA pH 8.0 + 10 mM KCl, which is a buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

Seraseq ctDNA Mutation Mix 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 150-200 bp. Variant allele frequency (VAF) and copy gain, is confirmed by digital PCR. VAF and CNV status is also measured by NGS as reported in the batch-specific CoA.

REAGENTS

Table 1. Seraseq ctDNA Mutation Mix v4

Material No.	Product
0710-3097	Seraseq ctDNA Mutation Mix v4 AF0.1%
0710-3099	Seraseq ctDNA Mutation Mix v4 AF0.5%
0710-3100	Seraseq ctDNA Mutation Mix v4 AF5%
0710-3101	Seraseq ctDNA Mutation Mix 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 Mutation Mix v4 and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq ctDNA Mutation Mix 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 Mutation Mix v4 is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 +10 mM KCl aqueous buffer.

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 Mutation Mix v4 frozen at -20 °C or colder. Once opened, a vial can be thawed and re-frozen up to five (5) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to five (5) or less. When stored in this fashion Seraseq ctDNA Mutation Mix v4 will be stable through the expiration indicated on the vial label.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq ctDNA Mutation Mix 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 Mutation Mix v4 is a mixture of human genomic DNA and synthetic DNA constructs that have been fragmented to a fragment size comparable to that of naturally occurring ctDNA with a fragment peak size of 150-220 bp. Seraseq ctDNA Mutation Mix v4 is formulated in a 1 mM Tris / 0.1 mM EDTA +10 mM KCl pH 8.0 buffer. 25 µL is provided per tube and the concentration is 10 ng/µL.

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 Mutation Mix v4 should be integrated into library preparation after the DNA isolation step. Refer to standard assay procedures in order to determine the amount of material to use.

Quality Control

Although Seraseq ctDNA Mutation Mix v4 is designed to 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 Mutation Mix v4 with each assay system prior to its routine use.

INTERPRETATION OF RESULTS

Detection of the variants within Seraseq ctDNA Mutation Mix 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 Mutation Mix 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.

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LIMITATIONS OF THE PROCEDURE
Seraseq ctDNA Mutation Mix 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 Mutation Mix 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 Mutation Mix v4 has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq ctDNA Mutation Mix 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	COSMIC ID
AKT1	c.49G>A	NM_005163.2	SNV	COSM33765
AR	c.2623C>T	NM_000044.6	SNV	COSM238555
ATM	c.1058_1059del	NM_000051.4	Deletion	COSM21924
BRAF	c.1799T>A	NM_004333.6	SNV	COSM476
BRCA1	c.1961del	NM_007294.4	Deletion	COSM219054
BRCA2	c.7934del	NM_000059.4	Deletion	COSM1738241
CDKN2A	c.9_32dup	NM_000077.5	Insertion	COSM13442
CHEK1	c.676del	NM_001114122.3	Deletion	COSM1352376*
CHEK2	c.1116_1117delinsGT	NM_007194.4	INDEL	COSM384945**
EGFR	c.2235_2249del	NM_005228.5	Deletion	COSM6223
EGFR	c.2303G>T	NM_005228.5	SNV	COSM6241
EGFR	c.2310_2311insGGT	NM_005228.5	Insertion	COSM12378
EGFR	c.2369C>T	NM_005228.5	SNV	COSM6240
EGFR	c.2389T>A	NM_005228.5	SNV	COSM6493937
EGFR	c.2573T>G	NM_005228.5	SNV	COSM6224
ERBB2	c.2313_2324dup	NM_004448.4	Insertion	COSM20959
ESR1	c.1613A>G	NM_000125.4	SNV	COSM94250
FGFR3	c.746C>G	NM_000142.5	SNV	COSM715
HRAS	c.182A>G	NM_005343.4	SNV	COSM499
HRAS	c.37G>C	NM_005343.4	SNV	COSM486
IDH1	c.394C>T	NM_005896.4	SNV	COSM28747
IDH2	c.419G>A	NM_002168.4	SNV	COSM41590
IDH2	c.515G>A	NM_002168.4	SNV	COSM33733
KIT	c.2361+67_2361+72delTTTTTT	NM_000222.3	Deletion	N/A
KIT	c.2447A>T	NM_000222.3	SNV	COSM1314

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Gene	Nucleotide Change	Transcript	Variant Type	COSMIC ID
KRAS	c.183A>C	NM_004985.5	SNV	COSM554
KRAS	c.34G>T	NM_004985.5	SNV	COSM516
KRAS	c.35G>A	NM_004985.5	SNV	COSM521
MAP2K1	c.370C>T	NM_002755.4	SNV	COSM235614
MAP4K3	c.246-2475_246-2470delTTTTTT	NM_003618.4	Deletion	N/A
MAP4K3	c.998-35_998-30delAAAAAA	NM_003618.4	Deletion	N/A
MET	c.3082+1del	NM_001127500.3	Deletion	COSM6947926
MLH1	c.232_243delinsATGTAAGG	NM_000249.4	INDEL	N/A
MSH2	c.1662-12_1677del	NM_000251.3	Deletion	N/A
MSH2	c.942+20_942+29delAAAAAAAAAA	NM_000251.3	Deletion	N/A
MSH6	c.2056_2060delinsCTTCTACCTCAAAAA	NM_000179.3	INDEL	N/A
MTOR	c.6644C>A	NM_004958.4	SNV	COSM20417
NF1	c.3738_3747del	NM_001042492.3	Deletion	COSM510741
NRAS	c.182A>G	NM_002524.5	SNV	COSM584
NTRK1	c.1783G>A	NM_002529.4	SNV	COSM9113104
NTRK2	c.1915G>A	NM_006180.6	SNV	N/A
NTRK3	c.1867G>A	NM_001012338.3	SNV	COSM6951362
PALB2	c.839del	NM_024675.4	Deletion	COSM1376815
PDGFRA	c.2525A>T	NM_006206.6	SNV	COSM736
PIK3CA	c.1633G>A	NM_006218.4	SNV	COSM763
PIK3CA	c.3140A>G	NM_006218.4	SNV	COSM775
PIK3CA	c.3203dup	NM_006218.4	Insertion	COSM249879
PIK3R1	c.1727_1729del	NM_181523.3	Deletion	COSM35737
PMS2	c.861_864del	NM_000535.7	Deletion	COSM5547641
PTCH1	c.2307_2308delinsTT	NM_000264.5	INDEL	COSM17587
PTEN	c.800del	NM_000314.8	Deletion	COSM5809
PTEN	c.741dup	NM_000314.8	Insertion	COSM4986
RAD51C	c.242C>A	NM_058216.3	SNV	N/A
RAD51C	c.338dup	NM_058216.3	SNV	N/A
RAD51D	c.271A>T	NM_002878.4	SNV	N/A
RAD51D	c.392dup	NM_002878.4	SNV	N/A
RAF1	c.770C>T	NM_002880.4	SNV	COSM181063
RB1	c.751C>T	NM_000321.3	SNV	COSM878
RET	c.2753T>C	NM_020975.6	SNV	COSM965
SLC7A8	c.-231_-224delTTTTTTTT	NM_012244.4	Deletion	N/A
SMARCB1	c.118C>T	NM_003073.5	SNV	COSM1002
STK11	c.734+1G>T	NM_000455.5	SNV	COSM51523
TERT	c.-124C>T	NM_198253.3	SNV	N/A

Gene	Nucleotide Change	Transcript	Variant Type	COSMIC ID
TERT	c.-146C>T	NM_198253.3	SNV	N/A
TP53	c.723del	NM_000546.6	Deletion	COSM6530
TP53	c.743G>A	NM_000546.6	SNV	COSM10662
TP53	c.818G>A	NM_000546.6	SNV	COSM10660
TSC1	c.1263+1G>T	NM_000368.5	SNV	COSM1738312
TSC2	c.2640-1G>A	NM_000548.5	SNV	COSM3361675
VHL	c.481C>T	NM_000551.4	SNV	COSM17612
ZNF2	c.*1525_*1530delTTTTTT	NM_021088.4	Deletion	N/A
CD74::NRG1	Intron 6::Intron 5	NM_001025159.3::NM_013964.5	Translocation	N/A
CD74::ROS1	Intron 6::Intron 34	NM_001025159.3::NM_001378902.1	Translocation	N/A
COL1A1::PDGFB	Intron 25::Intron 1	NM_000088.3::NM_002608.3	Translocation	N/A
EML4::ALK	Intron 13::Intron 19	NM_019063.5::NM_004304.5	Translocation	N/A
ETV6::NTRK3	Intron 5::Intron 14	NM_001987.5::NM_002530.4	Translocation	N/A
FGFR2::BICC1	Intron 17::Intron 2	NM_000141.5::NM_001080512.3	Translocation	N/A
FGFR3::TACC3	Exon 18::Intron 7	NM_000142.5::NM_006342.3	Translocation	N/A
NCOA4::RET	Intron 7::Intron 11	NM_001145263.2::NM_020975.6	Translocation	N/A
PML::NTRK2	Intron 2::Intron 12	NM_002675.4::NM_006180.6	Translocation	N/A
TPM3::NTRK1	Intron 7::Intron 9	NM_153649.4::NM_002529.4	Translocation	N/A
AKT2	Amplification	19:40736224_40791252	CNV	N/A
CCND1	Amplification	NM_053056.3:11:69455924_69469242	CNV	N/A
CCNE1	Amplification	NM_001238.4:19:30302898_30315219	CNV	N/A
CDK4	Amplification	NM_000075.4:58141510_58146093	CNV	N/A
ERBB2	Amplification	NM_004448.4:17:37844347_37884911	CNV	N/A
FGF19	Amplification	NM_005117.3:11:69513006_69518790	CNV	N/A
FGF3	Amplification	NM_005247.4:11:69624736_69634184	CNV	N/A
FGF4	Amplification	NM_002007.4:69587797_69590109	CNV	N/A
FGFR1	Amplification	NM_023110.3:8:38268661_38326153	CNV	N/A
MET***	Amplification	NM_001127500.3:7:116312250_116438431	CNV	N/A
MYC	Amplification	NM_002467.6:8:128747680_128755197	CNV	N/A
MYCN	Amplification	NM_005378.6:2:16080672_16087126	CNV	N/A

* COSMIC uses transcript ENST00000427383.6
** COSMIC uses transcript ENST00000328354
*** 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.