Seraseq™

ctDNA Reference Material v2
AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT

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

Seraseq™ ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT is formulated for use with targeted Next Generation Sequencing (NGS) assays that detect cancer-relevant somatic mutations present in the blood stream as circulating cell-free tumor DNA. This product is intended as a quality reference material for translational and disease research testing to monitor DNA extraction, library preparation, sequencing, and variant detection under a given set of bioinformatics pipeline parameters. *For Research Use Only. Not for use in diagnostic procedures.*

REAGENTS

Table 1. Different variant allele frequencies (AF) for Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT. Each Item No. is available as an individual product. Information in this Package Insert applies to all 6 of these products.

Item No.	Product
0710-0203	Seraseq™ ctDNA Reference Material v2 AF2%
0710-0204	Seraseq™ ctDNA Reference Material v2 AF1%
0710-0205	Seraseq™ ctDNA Reference Material v2 AF0.5%
0710-0206	Seraseq™ ctDNA Reference Material v2 AF0.25%
0710-0207	Seraseq™ ctDNA Reference Material v2 AF0.125%
0710-0208	Seraseq™ ctDNA Reference Material v2 WT

For all products: 1 vial, 5 mL per vial, 25 ng/mL concentration.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT as though it is capable of transmitting infectious agents. This product is formulated using a reference 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).

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 with 0.5% sodium hypochlorite solution. Dispose of all specimens and materials used in testing as though they contain infectious agents.

Handling Precautions

Do not use Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT at 2-8 °C. Do not freeze. Product expiry date is indicated on the vial and box labels.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq ctDNA Mutation Mix v2 is a mixture of human genomic DNA and synthetic DNA constructs. It should appear as a clear to pale yellow 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 v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT consists of DNA purified from a reference cell line, GM24385, plus constructs containing variants mixed at a defined allele frequency. Processing of the purified DNA is used to produce an average DNA fragment size of approximately 170 basepairs (Figure 1). The DNA is stabilized and introduced into a dilution of SeraCare's SeraCon™ Matribase to a concentration of ~25 ng/mL as determined using Thermo Fisher Qubit™ dsDNA BR Assay Kit. Material must undergo extraction prior to input into NGS library preparation. For different combinations of commonly-used, commercially-available extraction and quantification methods (including automated extraction workflows), ~80% average recovery was observed relative to the reference methods QIAGEN QIAamp® Circulating Nucleic Acid Kit (extraction) and Qubit dsDNA BR Assay Kit (quantification). 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

Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT may be input into workflows in a manner consistent with plasma fractions prior to extraction. Mix by vortexing to ensure a homogenous mixture before use. Following extraction, Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT may be processed through library preparation and sequencing in parallel with test specimens. Refer to your usual assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

Table 2 indicates each of the somatic mutations represented in Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT. Detection of mutations may differ across different NGS panels and different test reagent lots. While the presence and frequency of each mutation in this product is confirmed during manufacture using functional NGS and/or digital PCR assays, there may be differences in observed allele frequencies due to assay characteristics. Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT does not have assigned values for allele frequencies of the mutations present in the product. Each laboratory must establish an assay-specific expected value for each mutation and each lot of Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT. 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 changes in bioinformatics pipeline parameters. Additional support documents are available online at www.seracare.com/oncology.

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LIMITATIONS OF THE PROCEDURE

Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS. *TEST PROCEDURES* provided by manufacturers must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. This product is offered for Research Use Only. Not for use in diagnostic procedures. Data are provided for informational purposes. SeraCare Life Sciences does not claim that others can duplicate test results exactly. Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT is not a calibrator and should not be used for assay calibration. Adverse shipping and/or storage conditions or use of outdated product may produce erroneous results.

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.

Figure 1. Representative DNA fragment size distribution for Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT

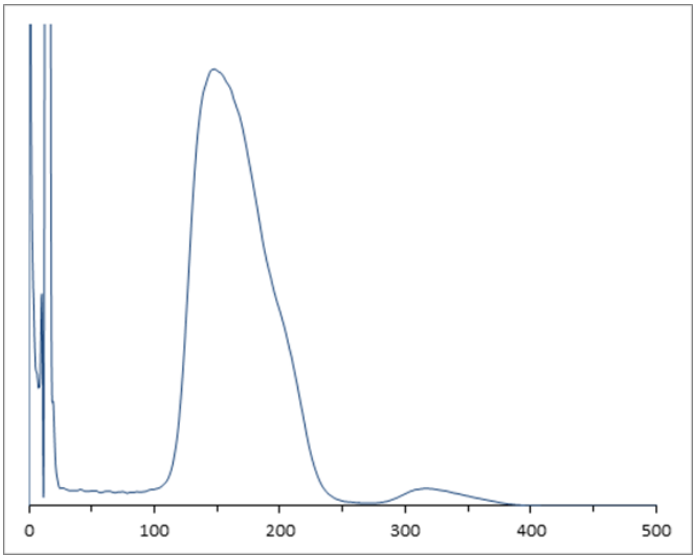

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Table 2. Somatic mutations present in Seraseq ctDNA Reference Material v2 AF2%, AF1%, AF0.5%, AF0.25%, AF0.125%, & WT

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid Change
AKT1	COSM33765	Substitution	c.49G>A	p.E17K
APC	COSM13127	Substitution	c.4348C>T	p.R1450*
APC	COSM18561	Insertion in HP 7N	c.4666_4667insA	p.T1556fs*3
ATM	COSM21924	Deletion	c.1058_1059delGT	p.C353fs*5
BRAF	COSM476	Substitution	c.1799T>A	p.V600E
CTNNB1	COSM5664	Substitution	c.121A>G	p.T41A
EGFR	COSM6224	SNV in 3N	c.2573T>G	p.L858R
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M
ERBB2	COSM682/20959	Insertion	c.2324_2325ins12	p.A775_G776insYVMA
FGFR3	COSM715	Substitution	c.746C>G	p.S249C
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y
FOXL2	COSM33661	Substitution	c.402C>G	p.C134W
GNA11	COSM52969	Substitution	c.626A>T	p.Q209L
GNAQ	COSM28758	SNV in HP 3N	c.626A>C	p.Q209P
GNAS	COSM27887	Substitution	c.601C>T	p.R201C
IDH1	COSM28747	Substitution	c.394C>T	p.R132C
JAK2	COSM12600	SNV in HP 3N	c.1849G>T	p.V617F
KIT	COSM1314	Substitution	c.2447A>T	p.D816V
KRAS	COSM521	Substitution	c.35G>A	p.G12D
MPL	COSM18918	Substitution	c.1544G>T	p.W515L
NCOA4-RET	N/A	Gene Fusion (DNA)	NCOA4{NC_000010.10}:r.1_1014+1312_RET{NC_000010.10}:r.2327-1437_5659	N/A
NPM1	COSM17559	Insertion	c.863_864insTCTG	p.W288fs*12
NRAS/CSDE1	COSM584	Substitution	c.182A>G	p.Q61R
PDGFRA	COSM736	Substitution	c.2525A>T	p.D842V
PDGFRA	COSM28053	Insertion	c.1694_1695insA	p.S566fs*6
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K
PIK3CA	COSM12464	Insertion	c.3204_3205insA	p.N1068fs*4
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R
PTEN	COSM4986	Insertion	c.741_742insA	p.P248fs*5
PTEN	COSM5809	Deletion 6N > 5N	c.800delA	p.K267fs*9
RET	COSM965	Substitution	c.2753T>C	p.M918T
SMAD4	COSM14105	Insertion	c.1394_1395insT	p.A466fs*28
TP53	COSM10648	Substitution	c.524G>A	p.R175H
TP53	COSM10660	Substitution	c.818G>A	p.R273H
TP53	COSM10662	Substitution	c.743G>A	p.R248Q
TP53	COSM6530	Deletion	c.723delC	p.C242fs*5
TP53	COSM18610	Deletion 5N >4N	c.263delC	p.S90fs*33
TPR-ALK	N/A	Gene Fusion (DNA)	TPR{NC_000001.10}:r.1_2185+246_ALK{NC_000002.11}:r.4125-550_6265	N/A