Seraseq™
Solid Tumor Mutation Mix-II (AF7) HC

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™ Solid Tumor Mutation Mix-II (AF7) HC is formulated for use with targeted Next Generation Sequencing (NGS) assays that detect mutations in key oncogenes and tumor suppressor genes. The Seraseq Solid Tumor Mutation Mix-II (AF7) HC is intended as a quality reference material for translational and disease research testing to monitor 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 provides added confidence in the reliability of results obtained for unknown specimens. The use of independent reference products may provide valuable information concerning assay sensitivity and bioinformatics pipeline analysis.

PRINCIPLES OF THE PROCEDURE
Seraseq Solid Tumor Mutation Mix-II (AF7) HC is ready to use in NGS assays in steps that follow DNA isolation; no further purification or DNA isolation is needed. The product contains human genomic DNA at a concentration of 25 ng/µL. The Reference Material is formulated in a diluted 1 mM Tris / 0.1mM EDTA pH 8.0 aqueous buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

REAGENTS
Item No. 0710-0073. 1 vial, 25 µL per vial, 25 ng/µL concentration.

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq Solid Tumor Mutation Mix-II (AF7) HC and all materials derived from human blood products as though they are capable of transmitting infectious agents. Seraseq Solid Tumor Mutation Mix-II (AF7) HC is manufactured using processed human genomic DNA and biosynthetic mutant sequences.

SAFETY PRECAUTIONS
Use Centers for Disease Control (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
Do not use Seraseq Solid Tumor Mutation Mix-II (AF7) HC beyond the expiration date. Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS
Store Seraseq Solid Tumor Mutation Mix-II (AF7) HC frozen at -20 ºC or colder. Once opened, a vial can be thawed and re-frozen up to ten (10) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to ten (10) or less.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION
Seraseq Solid Tumor Mutation Mix-II (AF7) HC 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 Solid Tumor Mutation Mix-II (AF7) HC is a mixture of human genomic DNA and synthetic DNA constructs in a 1mM Tris / 0.1mM EDTA pH 8.0 aqueous buffer. 25 µL is provided per tube and the concentration is 25 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 Solid Tumor Mutation Mix-II (AF7) HC should be integrated into library preparation after the DNA isolation step. Seraseq Solid Tumor Mutation Mix-II (AF7) HC must go through target selection and library preparation in parallel with the test specimens. Refer to your usual assay procedures in order to determine the amount of material to use.

Quality Control
Seraseq Solid Tumor Mutation Mix-II (AF7) HC does not have assigned values for the variant allele frequencies. However, the product is formulated using digital PCR quantitation to target each variant listed in Table 1 to be present at 7%. There are many reasons why assays may observe deviation from this target, which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of each lot of Seraseq Solid Tumor Mutation Mix-II (AF7) HC with each assay system prior to its routine use.

INTERPRETATION OF RESULTS
Detection of variants and the variant allele frequency may vary with different NGS targeted sequencing-based cancer panels and different test reagent lots. Since the reference material does not have an assigned value, the laboratory must establish an acceptable range for each variant and each lot of Seraseq Solid Tumor Mutation Mix-II (AF7) HC. 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. Support documents containing the target sequence coordinates are available online at http://www.seracare.com/oncology.html.

LIMITATIONS OF THE PROCEDURE
Seraseq Solid Tumor Mutation Mix-II (AF7) HC 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. Seraseq Solid Tumor Mutation Mix-II (AF7) HC is not a calibrator and should not be used for assay calibration. These materials are also not whole process controls and do not evaluate the methods used for specimen extraction.

Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

EXPECTED RESULTS
Specific detection of cancer variants and variant allele frequencies will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory should establish its own range of acceptable values. 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 1 lists mutations that are present in the product. Note that the GM24385 human cell line contains a heterozygous HRAS mutation (COSM249860) and heterozygous KIT mutation (COSM28026) that will be detected (depending on the assay utilized) at approximately 50%.

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SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Solid Tumor Mutation Mix-II (AF7) HC has been designed for use with targeted NGS Cancer hotspot panels for the purposes of assessing assay characteristics. The product is manufactured from purified human genomic DNA as well as biosynthetic DNA. Although the product is formulated with a 7% variant allele target for each mutation listed in Table 1 as determined by droplet digital PCR, Seraseq Solid Tumor Mutation Mix-II (AF7) HC 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-Second Edition. NCCLS document C24-A2, 1999.

Table 1: Seraseq Solid Tumor Mutation Mix-II (AF7) mutations

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

Note: List of mutations included in the Seraseq Solid Tumor Mutation Mix-II (AF7). The presence of the mutation in a particular assay depends upon the enrichment strategy and sequencing platform used. The mutation types are listed; HP = homopolymer, N = nucleotide