

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™ Tri-Level Tumor Mutation DNA Mix v2 is formulated for use with targeted Next Generation Sequencing (NGS) assays that detect mutations in key oncogenes and tumor suppressor genes. The Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 materials may provide valuable information concerning assay sensitivity and bioinformatics pipeline analysis.

PRINCIPLES OF THE PROCEDURE

Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 / 0.1 mM EDTA pH 8.0 aqueous buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

REAGENTS

Item No. 0710-0097. 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 Tri-Level Tumor Mutation DNA Mix v2 and all materials derived from human blood products as though they are capable of transmitting infectious agents. Seraseq Tri-Level Tumor Mutation DNA Mix v2 is manufactured using processed human genomic DNA and biosynthetic mutant sequences.

SAFETY PRECAUTIONS

Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens¹. 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 Tri-Level Tumor Mutation DNA Mix v2 beyond the expiration date. Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS

Store Seraseq Tri-Level Tumor Mutation DNA Mix v2 frozen at -20 °C or colder. Once opened, a vial can be thawed and re-frozen up to twelve (12) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to twelve (12) or less.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 Tri-Level Tumor Mutation DNA Mix v2 is a mixture of human genomic DNA and synthetic DNA constructs in a 1 mM Tris / 0.1 mM 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 Tri-Level Tumor Mutation DNA Mix v2 should be integrated into library preparation after the DNA isolation step. Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 Tri-Level Tumor Mutation DNA Mix v2 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 either 10%, 7% or 4%. There are many reasons why assays may observe deviation from these targets, which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of each lot of Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 Tri-Level Tumor Mutation DNA Mix v2. 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 Tri-Level Tumor Mutation DNA Mix v2 **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 Tri-Level Tumor Mutation DNA Mix v2 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 across 20 runs². Table 1 lists mutations and their target allele frequencies 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%.

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 target allele frequency for each mutation listed in Table 1 as determined by droplet digital PCR, Seraseq Tri-Level Tumor Mutation DNA Mix v2 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 Tri-Level Tumor Mutation DNA Mix v2 mutations and their target allele frequencies (AF)

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

Note: List of mutations and target allele frequencies (AF) included in the Seraseq Tri-Level Tumor Mutation DNA Mix v2. 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. Because of ambiguity surrounding exact genomic coordinates for sequence deletions contained entirely within repetitive motifs such as homopolymers, analytic calls generated by certain analyses may differ relative to the mutation names presented in this table. In such cases, additional analysis would be required during concordance evaluation.