Seraseq™

Solid Tumor Mutation Mix-I (AF1-10) 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-I (AF1-10) is a five member panel intended to challenge the lower limits of detection of Next Generation Sequencing assays that detect mutations in key oncogenes and tumor suppressor genes. The Seraseq Solid Tumor Mutation Mix-I (AF1-10) 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.*

PRODUCT DESCRIPTION
Seraseq Solid Tumor Mutation Mix-I (AF1-10) is a mixture of human genomic DNA and synthetic DNA constructs in a 1mM Tris / 0.1mM EDTA pH 8.0 buffer. Twenty-five (25) µL is provided per tube and the DNA concentration is 25 ng/µL. Five tubes are provided per kit, and the tubes represent a range of low level variant allele frequencies. Seraseq Solid Tumor Mutation Mix-I (AF1-10) is ready to use in Next Generation Sequencing (NGS) assays in steps that follow DNA isolation; no further purification or DNA isolation is needed.

REAGENTS
Item No. 0710-0001. 5 vials, 25 µL per vial, 25 ng/µL concentration.

Name	Variant Allele Frequency Target
AF10	10%
AF8	8%
AF5	5%
AF3	3%
AF1	1%

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq Solid Tumor Mutation Mix-I (AF1-10) and all materials derived from human blood products as though they are capable of transmitting infectious agents. Seraseq Solid Tumor Mutation Mix-I (AF1-10) is manufactured using genomic DNA extracted from 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). Purified genomic DNA is formulated in a 1 mM Tris / 0.1mM EDTA pH 8.0 aqueous buffer.

Safety Precautions
Use Center 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
Do not use Seraseq Solid Tumor Mutation Mix-I (AF1-10) beyond the expiration date. Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS
Store Seraseq Solid Tumor Mutation Mix-I (AF1-10) frozen at -20 ˚C or colder. Once opened, a vial can be thawed and re-frozen up to ten (10) times. Sub-aliquoting of the product in low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to ten (10) or less. Alterations in physical appearance may indicate instability or deterioration. Solutions that are visibly turbid should be discarded.

PROCEDURE
Materials Provided
Seraseq Solid Tumor Mutation Mix-I (AF1-10) is a mixture of human genomic DNA and synthetic DNA constructs in a 1mM Tris / 0.1mM EDTA pH 8.0 buffer. Twenty-five (25) µL is provided per tube, 5 tubes of varying allele frequency are including 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(s) to come to room temperature before use. Mix by vortexing to ensure a homogeneous solution and spin briefly. Seraseq Solid Tumor Mutation Mix-I (AF1-10) should be integrated into library preparation after the DNA isolation step. Seraseq Solid Tumor Mutation Mix-I (AF1-10) must go through the target selection and library preparation. Refer to your usual assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS AND INTERPRETATION
Detection of variants and the variant allele frequency may vary with different NGS targeted, sequencing-based cancer panels and different test reagent lots. Although the product does not have assigned values, it is tested using droplet digital PCR to verify variant allele frequencies for select mutations (Table1). There are many reasons why results from NGS assays may differ from digital PCR analysis, as these methods use different chemistry and have different levels of sensitivity and observed consistency.

Table 2 indicates the cancer relevant mutations 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 in Seraseq Solid Tumor Mutation Mix-I at approximately 50%.

Each mutation in Table 2 has an engineered 6-base pair Internal Quality Marker inserted nearby (within 25 bases). For example, the BRAF V600E mutation is shown here, the c.1799T>A labeled with an asterisk.

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CTCCATCGAGATTTCTCTGTACATCGAGCTAGACCAAAATC
|||||||||||||||*||||<6-bp>||||||||||||||
CTCCATCGAGATTTCACTGT------AGCTAGACCAAAATC
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More information about the Internal Quality Marker is available at http://www.seracare.com/oncology

LIMITATIONS OF THE PROCEDURE
Seraseq Solid Tumor Mutation Mix-I (AF1-10) 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.

SPECIFIC PERFORMANCE CHARACTERISTICS
Seraseq Solid Tumor Mutation Mix-I (AF1-10) has been designed for use with targeted NGS Cancer hotspot panels for the purposes of assessing assay characteristics and lower limits of detection.

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SPECIFIC PERFORMANCE CHARACTERISTICS – Cont'd

The product is manufactured from purified human genomic DNA as well as biosynthetic DNA. Although the product is formulated with a 10%, 8%, 5%, 3% or 1% variant allele target for each mutation listed in Table 1 as determined by droplet digital PCR, Seraseq Solid Tumor Mutation Mix-I (AF1-10) 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. CDC Recommendations for prevention of HIV transmission in health care settings. MMWR 36 (supp.2), 1987.

Table 1: Digital PCR Analysis of Variant Allele Frequencies in Seraseq Solid Tumor Mutation Mix-I (AF1-10)

	BRAF (COSM476) p.V600E	MPL (COSM18918) p.W515L	NPM1 (COSM17559) p.W288fs*12	PIK3CA (COSM775) p.H1047R	EGFR (COSM6225) p.E746_A750delELREA	KRAS (COSM521) p.G12D	Average across six mutations
AF1	1.36	1.01	1.68	1.08	1.08	0.93	1.19
AF3	2.93	2.87	3.00	2.90	2.67	2.75	2.85
AF5	4.47	4.43	4.47	4.43	4.67	4.05	4.42
AF8	7.67	7.83	8.60	7.87	7.67	7.49	7.85
AF10	9.93	10.03	11.34	10.33	10.37	9.97	10.33

Values given are the average variant allele frequencies of three (3) replicates

Table 2: Seraseq Solid Tumor Mutation Mix-I (AF1-10) mutations

	Gene	COSMIC ID of Mutation	Position (hg19)	CDS	Mutation Type	Amino Acid Change	Ion AmpliSeq Cancer Hotspot v2	Illumina TruSeq Cancer Panel
1	EGFR	COSM6224	55259515	c.2573T>G	SNV (Homopolymer)	p.L858R	●	○
2	FGFR3	COSM715	1803568	c.746C>G	SNV (Homopolymer)	p.S249C	●	⊙
3	GNAQ	COSM28758	80409488	c.626A>C	SNV (Homopolymer)	p.Q209P	●	⊙
4	AKT1	COSM33765	105246551	c.49G>A	SNV (Homopolymer)	p.E17K	●	●
5	ATM	COSM21924	108117846	c.1058_1059delGT	Small Deletion	p.C353fs*5	●	○
6	SMAD4	COSM14105	48603093	c.1394_1395insT	Small Insertion	p.A466fs*28	●	●
7	NPM1	COSM17559	170837547	c.863_864insTCTG	Large Insertion	p.W288fs*12	●	●
8	EGFR	COSM6225	55242465	c.2236_2250del15	Large Deletion	p.E746_A750delELREA	●	●
9	BRAF	COSM476	140453136	c.1799T>A	SNV	p.V600E	●	●
10	KRAS	COSM521	25398284	c.35G>A	SNV	p.G12D	●	●
11	PIK3CA	COSM775	178952085	c.3140A>G	SNV	p.H1047R	●	●
12	PIK3CA	COSM763	178936091	c.1633G>A	SNV	p.E545K	●	●
13	NRAS	COSM584	115256529	c.182A>G	SNV	p.Q61R	●	●
14	TP53	COSM10648	7578406	c.524G>A	SNV	p.R175H	●	●
15	CTNNB1	COSM5664	41266124	c.121A>G	SNV	p.T41A	●	●
16	IDH1	COSM28747	209113113	c.394C>T	SNV	p.R132C	●	●
17	EGFR	COSM6240	55249071	c.2369C>T	SNV	p.T790M	●	●
18	MPL	COSM18918	43815009	c.1544G>T	SNV	p.W515L	●	●
19	APC	COSM13127	112175639	c.4348C>T	SNV	p.R1450*	●	●
20	FLT3	COSM783	28592642	c.2503G>T	SNV	p.D835Y	●	●
21	PDGFRA	COSM736	55152093	c.2525A>T	SNV	p.D842V	●	●
22	RET	COSM965	43617416	c.2753T>C	SNV	p.M918T	●	⊙
23	GNAS	COSM27887	57484420	c.601C>T	SNV	p.R201C	●	○
24	TP53	COSM10662	7577538	c.743G>A	SNV	p.R248Q	●	⊙
25	KIT	COSM1314	55599321	c.2447A>T	SNV	p.D816V	●	○
26	JAK2	COSM12600	5073770	c.1849G>T	SNV	p.V617F	●	⊙

Note: The 26 mutations listed above have been observed to appear at the expected allelic frequency using the Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Torrent PGM™ Sequencing system. Illumina TruSeq Cancer panel primer locations in some of the variants (as indicated) will result in reduced perceived allelic frequency or non-observable mutation for that assay. Performance validation on assays other than the two noted above will be required.

Legend:	●	Mutation observed to appear at the expected allelic frequency	⊙	Mutation observed at reduced allelic frequency due to assay primer consideration	○	Mutation not observed due to assay primer consideration

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