Seraseq®

ctDNA Complete™ Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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

The Seraseq® ctDNA Complete™ Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT is a reference material 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 library preparation, sequencing, and variant detection under a given set of bioinformatics pipeline parameters. This product is *For Research Use Only. Not for use in diagnostic procedures.*

REAGENTS

Table 1. Different variant allele frequencies (AF) for Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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
0710-0669	Seraseq ctDNA Complete Reference Material AF5%
0710-0670	Seraseq ctDNA Complete Reference Material AF2.5%
0710-0671	Seraseq ctDNA Complete Reference Material AF1%
0710-0672	Seraseq ctDNA Complete Reference Material AF0.5%
0710-0673	Seraseq ctDNA Complete Reference Material AF0.1%
0710-0674	Seraseq ctDNA Complete Reference Material 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT at 2-8 °C. Do not freeze. Shelf life when stored under these conditions is two years from date of manufacture.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT is derived from DNA purified from a reference cell line, GM24385, plus constructs containing variants mixed at a defined allele frequency. Purified DNA is utilized to produce an average DNA fragment size of approximately 170 base pairs (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. QIAGEN QIAamp® Circulating Nucleic Acid Kit with carrier RNA (extraction) and Qubit dsDNA BR Assay Kit (quantification) were utilized to extract ctDNA from a 1mL volume, in triplicate (each AF), and yielded ~ 25-35 ng/mL of ctDNA. Note: 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT. Detection of mutations may differ across different NGS panels and different test reagent lots. While the presence and frequency of each mutation and amplification in this product is evaluated during manufacture using functional NGS and/or digital PCR assays, there may be differences in observed allele frequencies due to assay characteristics. Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT does not have assigned values for allele frequencies of the mutations and amplifications present in the product. Each laboratory must establish assay-specific expected values for each lot of Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT. Table 3 specifies region of the MET gene that may be present at higher levels than the rest of the MET gene. 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 Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & 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. Note that based on your particular assay protocol and regions interrogated, variants other than the 22 annotated in this product may be detected at varying allele frequencies. Seraseq ctDNA Complete Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT is not a calibrator and should not be used for assay calibration. Adverse shipping and/or storage conditions or use of expired 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. A representative DNA fragment sizing for Seraseq® ctDNA Complete™ Reference Material AF5% versus natural circulating cell-free DNA (ccfDNA)

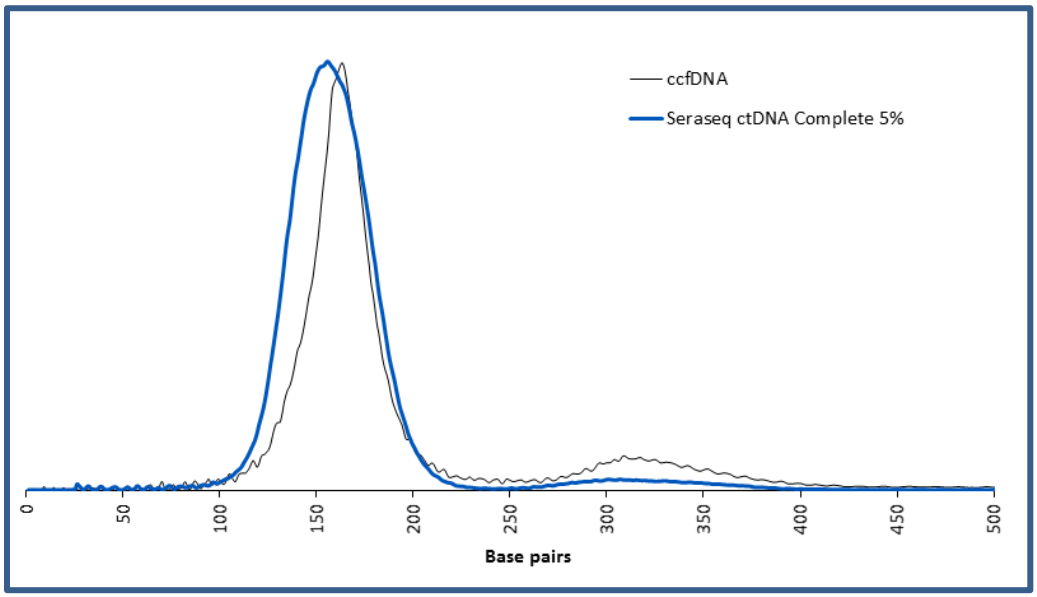

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Table 2. Somatic mutations present in Seraseq® ctDNA Complete™ Reference Material AF5%, AF2.5%, AF1%, AF0.5%, AF0.1%, & WT

#	Gene	Gene ID	AA Change	HGVS	Variant Type
1	AKT1	COSM33765	p.E17K	c.49G>A	SNV
2	ALK	COSM144250	p.G1202R	c.3604G>A	
3	ALK	COSM28055	p.F1174L	c.3522C>A	
4	BRAF	COSM476	p.V600E	c.1799T>A	
5	EGFR	COSM6240	p.T790M	c.2369C>T	
6	EGFR	COSM6224	p.L858R	c.2573T>G	
7	KIT	COSM1314	p.D816V	c.2447A>T	
8	KRAS	COSM516	p.G12C	c.34G>T	
9	KRAS	COSM521	p.G12D	c.35G>A	
10	KRAS	COSM554	p.Q61H	c.183A>C	
11	NRAS	COSM584	p.Q61R	c.182A>G	
12	PIK3CA	COSM775	p.H1047R	c.3140A>G	
13	BRCA1	COSM1383519	p.K654fs*47	c.1961delA	Del
14	EGFR	COSM12370	p.L747_P753>S	c.2240_2257del18	
15	BRCA2	COSM1738242	p.R2645fs*3	c.7934delG	
16	EGFR	COSM6256	p.S752_I759 del SPKANKEI	c.2254_2277del24	
17	EGFR	COSM6223	p.E746_A750 del ELREA	c.2235_2249del15	
18	PIK3CA	COSM12464	p.N1068fs*4	c.3204_3205insA	Ins
19	ERBB2	COSM20959	p.A775_G776 ins YVMA	c.2324_2325ins12	
20	ERBB2	N/A	Amplification	N/A	CNV
21	MET	N/A	Amplification		
22	MYC	N/A	Amplification		
23	CD74-ROS1	N/A	Translocation	N/A	Translocation
24	EML4-ALKv1	N/A	Translocation		
25	NCOA4-RET	N/A	Translocation		

Table 3. Region that may be present at two times the extra copies of the rest of MET

Gene	chr (hg19)	start	end	size (bp)
MET	chr7	116,372,111	116,418,397	46,286