

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 Reference Material WT is a reference material formulated for use with PCR (qPCR or dPCR) or 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

Material No. 0710-0720. 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 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 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 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 WT beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq ctDNA Reference Material 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 Reference Material WT is derived from DNA purified from a reference cell line (GM24385). Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Provided

Seraseq ctDNA Reference Material WT is derived from DNA purified from a reference cell line, GM24385. 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 1 mL 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 Reference Material 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 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 no detectable somatic mutation in the Seraseq ctDNA Reference Material WT. Each laboratory must establish assay-specific expected values for each lot of Seraseq ctDNA Reference Material 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.

LIMITATIONS OF THE PROCEDURE

Seraseq ctDNA Reference Material 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 4 annotated in this product may be detected at varying allele frequencies. Seraseq ctDNA Reference Material 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. DNA fragment sizing for Seraseq® ctDNA Reference Material WT (blue) and native ccfDNA (red)

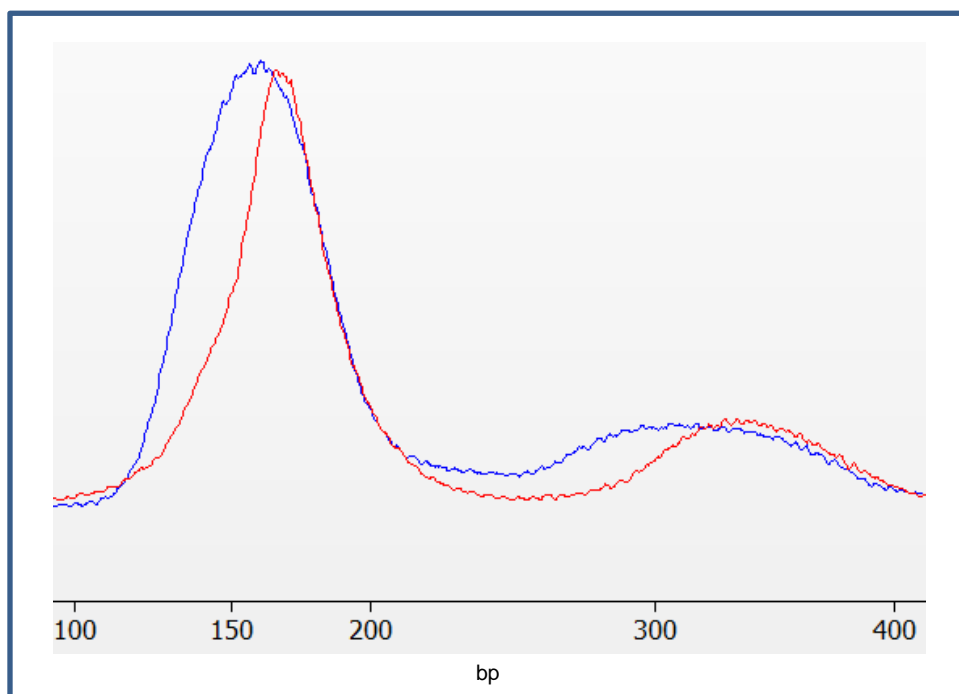


Table 2. Somatic mutations not detectable in the Seraseq® ctDNA Reference Material WT.

Material No.	Product	Gene	Gene ID	Amino Acid Change	Human Genome Variation Society (HGVS) Nomenclature	Variant Type	Target Allele Freq. (AF)
0710-0720	Seraseq ctDNA Reference Material WT	EGFR	COSM6240	p.T790M	c.2369C>T	SNV	0%
		EGFR	COSM6224	p.L858R	c.2573T>G	SNV	0%
		EGFR	COSM6225	p.E746_A750 delELREA	c.2236_2250del15	DEL	0%
		EGFR	COSM6252	p.G719S	c.2155G>A	SNV	0%
		KRAS	COSM521	p.G12D	c.35G>A	SNV	0%
		BRAF	COSM476	p.V600E	c.1799T>A	SNV	0%