

**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<sup>®</sup> ctDNA EGFR Panel Reference Material AF1% and AF0.1% are reference materials 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 EGFR-based 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. Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1%.

Material No.	Product
0710-0718	Seraseq ctDNA EGFR Panel Reference Material AF1%
0710-0719	Seraseq ctDNA EGFR Panel Reference Material AF0.1%

Each Material No. is available as an individual product. Information in this Package Insert applies to both products.

**WARNINGS AND PRECAUTIONS**

*For Research Use Only. Not for use in diagnostic procedures.*  
**CAUTION:** Handle Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1% 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<sup>1</sup>. 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 EGFR Panel Reference Material AF1% and AF0.1% beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

**STORAGE INSTRUCTIONS**

Store Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1% 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 EGFR Panel Reference Material AF1% and AF0.1% 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 EGFR Panel Reference Material AF1% and AF0.1% 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<sup>™</sup> Matribase to a concentration of ~25 ng/mL as determined using Thermo Fisher Qubit<sup>™</sup> dsDNA BR Assay Kit. Material must undergo extraction prior to input into NGS library preparation. QIAGEN QIAamp<sup>®</sup> 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 EGFR Panel Reference Material AF1% and AF0.1% 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 EGFR Panel Reference Material AF1% and AF0.1% 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 EGFR Panel Reference Material AF1% and AF0.1%. 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 a digital PCR assay, there may be differences in observed allele frequencies due to assay characteristics. Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1% do not have assigned values for allele frequencies of the mutations of the amplified gene in the product. Each laboratory must establish assay-specific expected values for each lot of Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1%. 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](http://www.seracare.com/oncology).

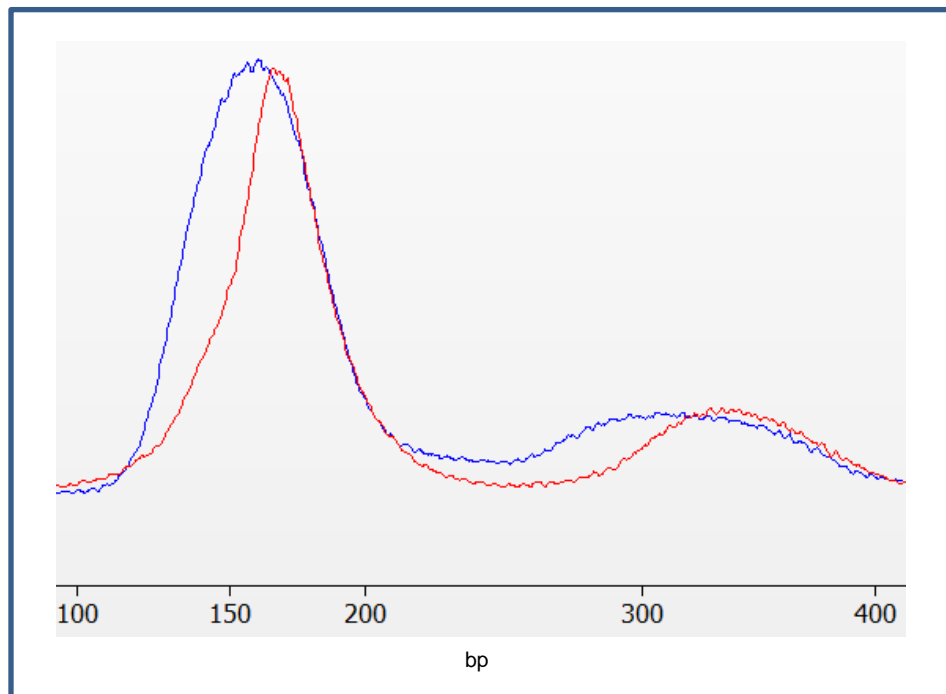
### LIMITATIONS OF THE PROCEDURE

Seraseq ctDNA EGFR Panel Reference Material AF1% and AF0.1% 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 EGFR Panel Reference Material AF1% and AF0.1% are not calibrators 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 EGFR Panel Reference Material AF1% (blue) and native ccfDNA (red)



**Table 2.** Somatic mutations present in Seraseq® ctDNA EGFR Panel Reference Material AF1% and AF0.1%.

Material No.	Product	Gene	Gene ID	Amino Acid Change	Human Genome Variation Society (HGVS) Nomenclature	Variant Type	Target Allele Freq. (AF)
0710-0718	Seraseq® ctDNA EGFR Panel Reference Material AF1%	EGFR	COSM6240	p.T790M	c.2369C>T	SNV	1%
		EGFR	COSM6224	p.L858R	c.2573T>G	SNV	1%
		EGFR	COSM6225	p.E746_A750 delELREA	c.2236_2250del	DEL	1%
		EGFR	COSM6252	p.G719S	c.2155G>A	SNV	1%
0710-0719	Seraseq® ctDNA EGFR Panel Reference Material AF0.1%	EGFR	COSM6240	p.T790M	c.2369C>T	SNV	0.1%
		EGFR	COSM6224	p.L858R	c.2573T>G	SNV	0.1%
		EGFR	COSM6225	p.E746_A750 delELREA	c.2236_2250del	DEL	0.1%
		EGFR	COSM6252	p.G719S	c.2155G>A	SNV	0.1%