Seraseq®
ctDNA ESR1 Mix WT, AF1%

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 ESR1 Mix is intended for use with Next Generation Sequencing (NGS) assays that identify variants present in circulating tumor DNA (ctDNA) present in the blood. The Seraseq ctDNA ESR1 Mix is intended as a quality reference material for translational and disease research testing and 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.*

SUMMARY
A well-designed quality control program can provide added confidence in the reliability of results obtained for unknown specimens. The use of independent reference products may provide valuable information concerning assay accuracy and bioinformatics pipeline analysis.

PRINCIPLES OF THE PROCEDURE
Seraseq ctDNA ESR1 Mix is ready-to-use in Next Generation Sequencing (NGS) assays in steps that follow DNA isolation; no further purification or DNA isolation is needed. The reference materials should follow the same workflow as unknown samples. The product contains DNA at a concentration of 10 ng/µL. The reference material is formulated in 1 mM Tris / 0.1 mM EDTA pH 8.0 +10 mM KCl, which is a buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

Seraseq ctDNA ESR1 Mutation Mix AF1% contains 20 mutations (not including those present in the GM24385 background) that are associated with ctDNA monitoring and are predominantly druggable mutations (see Table 2). The product is formulated to simulate ctDNA fragment sizes with a peak between 150-220 bp. Variant allele frequency (VAF) and copy gain, is confirmed by digital PCR. VAF is also measured by NGS as reported in the batch-specific TPR.

REAGENTS
Table 1. Seraseq ctDNA ESR1 Mixes

Material No.	Product
0710-3565	Seraseq ctDNA ESR1 Mutation Mix AF1%
0710-3564	Seraseq ctDNA ESR1 Mix WT

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

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq ctDNA ESR1 Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq ctDNA ESR1 Mix is manufactured using genomic DNA extracted from the 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). Seraseq ctDNA ESR1 Mix is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 + 10mM KCl aqueous buffer.

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 up with 0.5% sodium hypochlorite solution. Dispose of all specimens and materials used in testing as though they contain infectious agents.

Handling Precautions
Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS
Store Seraseq ctDNA ESR1 Mix frozen at -20 °C or colder. Once opened, a vial can be thawed and re-frozen up to five (5) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to five (5) or less. When stored in this fashion Seraseq ctDNA ESR1 Mix will be stable through the expiration indicated on the vial label.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION
Seraseq ctDNA ESR1 Mix 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 ESR1 Mix is a mixture of human genomic DNA and synthetic DNA constructs that have been fragmented to a fragment size comparable to that of naturally occurring ctDNA with a fragment peak size of 150-220 bp. Seraseq ctDNA ESR1 Mix is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 + 10 mM KCl buffer. 25 µL is provided per tube and the concentration is 10 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 ctDNA ESR1 Mix should be integrated into library preparation after the DNA isolation step. Refer to standard assay procedures in order to determine the amount of material to use.

Quality Control
Although Seraseq ctDNA ESR1 Mix is designed to ctDNA present at the indicated target VAF, the product does not have assigned values for mutation frequencies. There are many reasons why assays may observe deviation from the representative data which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of Seraseq ctDNA ESR1 Mix with each assay system prior to its routine use.

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14418-01 May 2024

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INTERPRETATION OF RESULTS
Detection of the variants within Seraseq ctDNA ESR1 Mix may vary with different types of tests and different test kit lots. Since the reference material does not have an assigned value, the laboratory must establish a range for each lot of Seraseq ctDNA ESR1 Mix. 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.

LIMITATIONS OF THE PROCEDURE
Seraseq ctDNA ESR1 Mix MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

TEST PROCEDURES and *INTERPRETATION OF RESULTS* provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. Seraseq ctDNA ESR1 Mix is not a calibrator and should not be used for assay calibration. Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

EXPECTED RESULTS
Specific detection of variants and variant allele frequencies will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory should establish its own acceptance criteria. For example, the acceptable range for each variant might include all values within two standard deviations of the mean of 20 data points obtained in 20 runs[2]. Table 1 lists the variants in the product.

SPECIFIC PERFORMANCE CHARACTERISTICS
Seraseq ctDNA ESR1 Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq ctDNA ESR1 Mix 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 2: List of Mutations

#	Gene	Nucleic acid change	Amino Acid Change	Variant Type
1	ESR1	c.1138G>C	E380Q	SNV
2		c.1387T>C	S463P	SNV
3		c.1603C>A	P535T	SNV
4		c.1607_1608delinsAT	L536H	INDEL
5		c.1607T>A	L536H	SNV
6		c.1607T>C	L536P	SNV
7		c.1607T>G	L536R	SNV
8		c.1607_1608delinsAG	L536Q	INDEL
9		c.1610_1611delinsCA	Y537S	INDEL
10		c.1609_1610delinsAG	Y537S	INDEL
11		c.1610A>C	Y537S	SNV
12		c.1609T>A	Y537N	SNV
13		c.1608_1609delinsTA	Y537N	INDEL
14		c.1610A>G	Y537C	SNV
15		c.1609T>G	Y537D	SNV
16		c.1613A>G	D538G	SNV
17	PIK3CA	c.1610_1615dupATGACC	D538_L539insHD	INDEL
18		c.1625A>G	E542G	SNV
19		c.1624G>A	E542K	SNV
20		c.1633G>A	E545K	SNV

NOTE: Above list does not include variants present in the GM24385 background. Indels are defined as deletion/insertions less than 10 base pairs.

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