Seraseq®

ctDNA Prostate Mix 1%, 0.5%, 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 Prostate 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 Prostate 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 Prostate 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 Prostate Mix contains 54 mutations (not including those present in the GM24385 background) that are associated with metastatic prostate cancer (Table 2). The product is formulated to simulate ctDNA fragment sizes with a peak between 150-220 bp. The variant allele frequency of the 54 introduced variants is confirmed by droplet digital PCR as well as measured by NGS.

REAGENTS

Table 1. Seraseq ctDNA Prostate Mixes

Material No.	Product
0710-3327	Seraseq® ctDNA Prostate Mix WT
0710-3328	Seraseq® ctDNA Prostate Mix AF1%
0710-3329	Seraseq® ctDNA Prostate Mix AF0.5%

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 Prostate Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq ctDNA Prostate 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). Purified genomic DNA is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 + 10 mM 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 Prostate 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.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq ctDNA Prostate 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 Prostate 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 Prostate Mix is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 + 10 mM KCl aqueous buffer. Twenty-five (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 Prostate Mix should be integrated into library preparation after the DNA isolation step. If a DNA shearing step is part of the workflow, Seraseq ctDNA Prostate 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 Prostate Mix is designed to simulate a ctDNA sample from a person with prostate cancer 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 Prostate Mix with each assay system prior to its routine use.

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14467-01 September 2024

INTERPRETATION OF RESULTS
Detection of the variants within Seraseq ctDNA Prostate 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 Prostate 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 Prostate 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 Prostate 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 2 lists the variants in the product and their target allele frequencies (verified by digital PCR).

SPECIFIC PERFORMANCE CHARACTERISTICS
Seraseq ctDNA Prostate Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq ctDNA Prostate 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– Fourth Edition. CLSI document C24, 2016.

Table 2: List of Mutations Incorporated

Genes	Nucleotide Change	Protein Change	Type of Alteration	Variant Size (bp)
APC	c.4348C>T	p.R1450*	SNV	1
APC	c.4666dup	p.T1556fs	Duplication	1
AR	c.2105T>A	p.L702H	SNV	1
AR	c.2623C>T	p.H875Y	SNV	1
AR	c.2632A>G	p.T878A	SNV	1
ATM	c.2543_2571del	p.E848fs	Deletion	29
ATR	c.2320dup	p.I774fs	Duplication	1
BARD1	c.1600_1634delinsGCG	p.T534fs	Indel	35
BRAF	c.1799T>A	p.V600E	SNV	1
BRCA1	c.3481_3491del	p.E1161fs	Deletion	11
BRCA2	c.1813dup	p.I605fs	Insertion	1
BRCA2	c.8954-8_9136del	Deletion	Deletion	284
BRIP1	c.2392C>T	p.R798*	SNV	1
CDK12	c.4382del	p.G1461fs	Deletion	1
CDKN2A	c.9_32dup	p.A4_P11dup	Insertion	24
CHEK1	c.676del	p.T226fs	Deletion	1
CHEK2	c.1116_1117delinsGT	p.K373*	Indel	2
FANCA	c.2778+1G>A	Splice variant	SNV	1
FANCL	c.1096_1099dup	p.T367fs	Duplication	4

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Genes	Nucleotide Change	Protein Change	Type of Alteration	Variant Size (bp)
KIT	c.2361+67_2361+72delTTTTTT	MSI BAT-25	Deletion (25T -> 19T)	6
KRAS	c.34G>T	p.G12C	SNV	1
MAP4K3	c.998-35_998-30delAAAAAA	MSI MONO-27	Deletion (27A -> 21A)	6
MAP4K3	c.246-2475_246-2470delTTTTTT	MSI MONO-27	Deletion (27A -> 21A)	6
MLH1	c.1852_1854del	p.K618del	Deletion	3
MRE11	c.1100_1131del	p.Val367fs	Deletion	32
MSH2	c.942+3A>T	Splice Variant	SNV	1
MSH2	c.942+20_942+29delAAAAAAAAAA	MSI BAT-26	Deletion (27A -> 17A)	10
MSH6	c.3261dup	p.F1088fs	Duplication	1
NBN	c.1396del	p.R466fs	Deletion	1
PALB2	c.1059_1077delinsGG	p.S354fs	Indel	19
PIK3CA	c.3140A>G	p.H1047R	SNV	1
PIK3R1	c.1727_1729del	p.T576del	Deletion	3
PMS2	c.2243_2246del	p.K748fs	Deletion	4
PTEN	c.741dup	p.P248fs	Insertion	1
PTEN	c.800del	p.K267fs	Deletion	1
RAD51B	c.321dup	p.G108fs	Duplication	1
RAD51C	c.706-2A>G	Splice Variant	SNV	1
RAD51D	c.694_715delinsTGAGAGCTGAAGACCCTGGCCT	p.R232*	Indel	22
RAD54L	c.636_637dup	p.K213fs	Duplication	2
RB1	c.751C>T	p.R251*	SNV	1
SLC7A8	c.-231_-224delTTTTTTTT	MSI NR-21	Deletion (21A -> 13A)	9
SPOP	c.44_47dup	p.P17fs	Duplication	4
TP53	c.743G>A	p.R248Q	SNV	1
ZNF2	c.*1525_*1530delTTTTTT	MSI NR-24	Deletion (23T -> 17T)	6

Translocation	5' Transcript	5' Breakpoint GRCh38	3' Transcript	3' Breakpoint GRCh38
SLC45A3::ETV1	NM_033102.3	1:205666186	NM_004956.5	7:13983465
TMPRSS2::ERG	NM_005656.4	21:41501890	NM_182918.4	21:38510697

CNV	GRCh38 Amplified Region
CCND1	11:69634261_69760196
MYC	8:127654539_127799653

NOTE: Above list does not include variants present in the GM24385 background. Substitution refers to single nucleotide variant; Indels are defined as deletion/insertions.

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