

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® Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are designed for use with assays designed to measure methylation at CpG sites of DNA. The Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are intended as quality reference materials for translational and disease research testing and monitors library preparation, sequencing, and methylation site 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

The Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are ready-to-use in digital PCR and 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 with 10mM KCl, which is a buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

The Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are formulated to have detectable CpG methylation at >90% of, or <2% of CpG sites, respectively. Detection is confirmed by digital PCR and reported in a batch-specific technical product report.

REAGENTS

Table 1. Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix

Material No.	Product
0710-3088	Seraseq Unmethylated ctDNA Mutation Mix
0710-3089	Seraseq Methylated ctDNA Mutation Mix

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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are 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>). The extracted DNA has been fragmented and methylated in the case of the methylated material, material number 0710-3089. The final ctDNA formulation is present in a 1 mM Tris / 0.1 mM EDTA pH 8.0 with 10mM KCl aqueous buffer.

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 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are a mixture of human genomic DNA that has been enzymatically treated and purified. 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are a mixture of human genomic DNA that has been enzymatically treated and purified in a 1 mM Tris / 0.1 mM EDTA pH 8.0 buffer. 25 μL is provided per vial 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix are designed to simulate varied methylation states, the product does not have assigned values for methylation status. 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix with each assay system prior to its routine use.

INTERPRETATION OF RESULTS

Detection of the variants within Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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 methylation 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 runs². Table 2 lists the percent methylation of a representative batch of material (measured by digital PCR).

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq Methylated ctDNA Mutation Mix & Unmethylated ctDNA Mutation 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: Percent of methylated DNA molecules at representative positions

SNP	Percent Methylation 0710-3088	Percent Methylation 0710-3089
CCND2	0.44	100
EGFR	0.74	99
ETV6	0.63	114
FANCA	0.78	95
MYB	1.1	103
RET	0.90	104
TFRC	0.70	90

NOTE: Taqman SNP Assays run on the BioRad QX-200 Droplet Digital PCR System. Data are from a representative batch, please see batch-specific Technical Product Report for specific values.