Seraseq®
gDNA MSI-High Mix

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® gDNA MSI-High Mix product is a reference material formulated for use with Next Generation Sequencing (NGS) assays that detect microsatellites in human cancer patient samples. This product is intended for use as a reference material in the determination of the number of extended microsatellites in a cancer patient sample analyzed by NGS assays under a given set of bioinformatics pipeline parameters. Product is *For Research Use Only. Not for use in diagnostic procedures.*

REAGENTS

0710-1670: Seraseq gDNA MSI-High Mix
1 vial, 25 ng/µl concentration, 20 µl fill volume, and 500 ng total mass.

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq gDNA MSI-High Mix product as though it is capable of transmitting infectious agents. This product consists of purified genomic DNA from a diseased breast cancer cell line.

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 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 gDNA MSI-High Mix product beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS
Store Seraseq gDNA MSI-High Mix frozen at -20°C. Aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze-thaw cycles. Shelf life when stored under these conditions is two years from date of manufacture.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION
Seraseq gDNA MSI-High Mix is formulated as a tumor only reference material, derived from expanded/cultured human cell line of a diseased (tumor) patient, and should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vial should be discarded.

PROCEDURE
Materials Provided
Seraseq gDNA MSI-High Mix consists of high molecular weight DNA purified from a human diseased cell line. The purified DNA is present in a 1 mM Tris, 0.1 mM EDTA, pH 8.0 aqueous buffer. Material is ready to use in NGS assays in steps that follow DNA isolation. No further purification or DNA isolation is needed.

Materials Required but not Provided
Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use
Thaw the product vial on ice. Mix by vortexing to ensure a homogenous solution and spin briefly. Seraseq gDNA MSI-High Mix may be input directly into library preparation following procedures used for clinical specimens. Refer to your assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS & INTERPRETATION OF RESULTS
Table 1 provides MSI analysis result for the Seraseq gDNA MSI-High Mix product as determined by the TSO500 assay. Detection of microsatellites may differ across different NGS panels, and concomitantly the MSI score and MSI-High determination for this product by other targeted NGS panels may differ. Each laboratory must establish an expected MSI score for the Seraseq gDNA MSI-High Mix product. 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.

LIMITATIONS OF THE PROCEDURE
Seraseq gDNA MSI-High Mix 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. Seraseq gDNA MSI-High Mix is not a calibrator and should not be used for assay calibration. This material is not a whole-process control and does not evaluate the method used for specimen extraction. Adverse shipping and/or storage conditions or use of outdated 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.

Table 1: MSI status determination for the Seraseq gDNA TMB Mix based on the TSO500 Assay.

Product Name	Material Number	Av. MSI Sites Detected*	Av. Unstable MSI sites*	Av. MSI Score*	MSI Call
Seraseq® gDNA MSI-High Mix	0710-1670	106	81	77.1	High

**MSI measurements are from replicate runs on the TSO500. MSI score is the ratio of the unstable MSI sites to the total number of sites detected (expressed as a percentage). The value must be >20% for an MSI-High result.*

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Seraseq® MSI Reference Materials

0710-1670 | 0710-1675 | 0710-1676

This product datasheet provides an overview of the analysis and verification of MSI status of the new Seraseq Microsatellite Instability (MSI) Reference Materials for molecular MSI assay LoD verification as well as confirmation of MSI-High status of patient samples analyzed by targeted NGS panels.

Seraseq MSI Reference Materials

Product Description	Kit Composition	Material No	Conc.	Volume	Total Mass (DNA)
Seraseq gDNA MSI-High Mix	gDNA - Tumor	0710-1670	25 ng/ µl	20 µl	500 ng
Seraseq MSI Reference Panel Mix AF5%	gDNA - Tumor gDNA - Normal	0710-1675	2 x 20 ng/ µl	2x 15 µl	2 x 300 ng
Seraseq MSI Reference Panel Mix AF20%	gDNA - Tumor gDNA - Normal	0710-1676	2 x 20 ng/ µl	2x 15 µl	2 x 300 ng

Seraseq MSI Reference Panels:

- Plasmid-based spike ins of clinically actionable MSI targets typically analyzed in PCR/NGS assays
- Provided as a tumor-normal (matched) set
- Genomic DNA mix in TE buffer
- Available as AF5% and AF20% blends
- MSI status determined by qPCR/CE and digital PCR analyses

Table 1: Targeted microsatellite markers in the Seraseq MSI Reference Panel products

Marker	Gene	Chromosome	Position (hg19 based)	Comment
BAT-25	KIT (intron16)	chr4	55598211	25T -> 19T
BAT-26	MSH2 (intron5)	chr2	47641559	27A -> 17A
NR-21	SLC7A8 (5'UTR)	chr14	23652346	21A -> 13A
NR-24	ZNF2 (3'UTR)	chr2	95849361	23T -> 17T
MONO-27[1]	MAP4K3 (intron 3)	chr2	39573062	27A -> 21A
	MAP4K3 (intron 13)		39536689	

[1]There is ambiguity in the literature on the MONO-27 locus so two constructs are included in the product to ensure compatibility (see, Bacher J, Halberg R, Kent-First M, Wood KV. "Methods and kits for detecting mutations" US Patent US20090068646A1 issued March 12, 2009; and Pino MS, Chung DC. "Application of molecular diagnostics for the detection of Lynch syndrome." Expert review of molecular diagnostics vol. 10,5 (2010): 651-65. doi:10.1586/erm.10.45).

Table 2: Quantitation (qPCR/CE and ddPCR) of MSI status of Seraseq® MSI Reference Panel AF5% & AF20%

MSI Marker	ddPCR			qPCR		
	AF5%	AF20%	WT (0%)	AF5%	AF20%	WT (0%)
BAT-25	4.1%	19.1%	0.0%	Unstable	Unstable	Stable
BAT-26	4.9%	17.9%	0.0%	Unstable	Unstable	Stable
NR-21	4.3%	18.5%	0.0%	Stable	Unstable	Stable
NR-24	4.2%	18.2%	0.0%	Unstable	Unstable	Stable
MONO-27	5.1%	19.9%	0.02%	Unstable	Unstable	Stable

Seraseq gDNA MSI-High Mix

- Human diseased cell-line (tumor-only)
- Genomic DNA in TE buffer
- Tumor-only NGS-based analysis

Verification of MSI-High status performed on two different targeted NGS assays – TSO500 (ILMN – Table 3) and Sophia STS (Sophia Genetics – Table 4).

Table 3: TSO500 assay analysis of MSI status of the Seraseq gDNA MSI-High Mix

Product Name	NGS Assay	Av. MSI Sites Detected*	Av. Unstable MSI sites*	Av. MSI Score*	MSI Call
Seraseq gDNA MSI-High Mix	TSO500	106	81	77.1	High

*MSI measurements are from replicate runs on the TSO500. MSI score is the ratio of the unstable MSI sites to the total number of sites detected (expressed as a percentage). The value must be >20% for an MSI-High result.

Table 4: Solid Tumor Solution (STS) determination of MSI status of the Seraseq gDNA MSI-High Mix

MSI locus	Local Score*	Global score*
BAT_25_FWD	3.8	3.8
BAT_25_REV	4.4	4.2
BAT_26_REV	10.6	9.8
CAT_25_REV	6.85	6.75
NR_21_FWD	2.5	2.75
NR_21_REV	0.3†	2.1†
NR_22_FWD	2.3	2.15
NR_22_REV	2	2.3
NR_27_REV	9.5†	8.4†
Overall	37.35	37.1
MS status	MSI-HC	

*MSI distance scores obtained with Sophia Genetics STS Library Prep Kit II and MSI module of Sophia DDM software v.5.8.3 are shown. Minimum overall distance score >14 is required for an MSI-HC (high confidence) status. Local scores calculated against unrelated run samples.

Acknowledgments

We thank the following laboratories for the PCR and NGS analysis data presented in this datasheet:

- TSO500 assay analysis performed by ILMN R&D (San Diego, CA)
- Digital PCR data analysis performed by Bio-Rad Laboratories Digital Biology R&D (Pleasanton, CA)
- qPCR/CE analysis using the Promega MSI Analysis v1.2 was performed at the Molecular Pathology laboratory, UCSF (San Francisco, CA)
- MSI analysis using Sophia Solid Tumor Solution (STS) assay was performed by Gen-ERA Diagnostics (Turkey)

CONTACT US

For additional information on the Seraseq MSI reference Materials as well as our immuno-oncology controls that includes tissue TMB and blood TMB reference Materials, please visit our tumor profiling product landing page at https://www.seracare.com/resources-and-education/tumor-profiling/ email us at infobox@seracare.com or call **+1-800-676-1881.**

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The package insert for this panel can be found at www.seracare.com.

A printed copy of the package insert or data sheet may be requested by email at info@seracare.com or by phone at 508.244.6400.

PRODUCT SHEET

Seraseq® Microsatellite Instability (MSI) Reference Materials

MICROSATELLITE INSTABILITY REFERENCE MATERIALS FOR DETECTION AND VALIDATION OF MSI BIOMARKERS IN CANCER PATIENT SAMPLES MEASURED BY PCR OR NGS.

HIGHLIGHTS

VALIDATE LOD OF MICROSATELLITE INSTABILITY (MSI) ASSAYS WITH GROUND-TRUTH MSI BIOMARKERS AT TWO AF LEVELS

APPLY PCR AND NGS TO QUANTITATE CANCER-ASSOCIATED MICROSATELLITES IN PATIENT SAMPLES

HIGH-QUALITY MANUFACTURED REFERENCE MATERIAL; PROVIDES CONSISTENT GROUND TRUTH

INTRODUCTION

Microsatellites are regions of DNA repeats with different lengths, i.e., instability, highlighting DNA mismatch repair gene deficiencies. Typical repeat units are between 1-6 base pairs and the number of repeats vary from person to person such that each person has a set length of these microsatellites in their genome. Measurements of MSI have traditionally been performed using qPCR/CE fragment length analysis methods, or immunohistochemistry (IHC), but new methodologies such as digital droplet PCR (ddPCR) and Next Generation Sequencing (NGS) are now being applied to determination of MSI status of cancer patients. High incidence of microsatellite instability (MSI) has been linked to favorable outcomes in immuno-oncology (I-O) treatment response by patients with diseases such as Lynch Syndrome and colorectal cancer. Hence, determination of MSI status for cancer patients is important in I-O therapeutics management.

LGC SeraCare has developed microsatellite instability (MSI) reference materials that support qPCR and NGS assays that target a range of short tandem repeat regions commonly analyzed for microsatellite instabilities. For assays that target specific mono and dinucleotide repeats such as BAT-25, BAT-26, NR-21, NR-24, MONO-27, we have created MSI reference materials containing these markers blended at two different allele frequency (AF) levels—5% and 20%. Additionally, for NGS MSI assays that analyze for a large number of microsatellite loci across the human genome, we have a human diseased cell line-based MSI-High reference material for such analysis. These products are quantitated by PCR (qPCR/CE and ddPCR) and by targeted NGS assays to support all product claims.

MICROSATELLITE BIOMARKERS AND GENOMIC LOCATIONS IN THE SERASEQ® MSI REFERENCE PANEL MIX AF5% AND AF20%

Marker	Gene	Chromosome	Position (hg19 based)	Comment
BAT-25	KIT (intron16)	chr4	55598211	25T -> 19T
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TARGETED NGS ASSAY DETERMINATION OF MICROSATELLITE INSTABILITY STATUS OF THE SERASEQ® gDNA MSI-HIGH MIX.

Product Name	NGS Assay	Av. MSI Sites Detected*	Av. Unstable MSI sites*	Av. MSI Score*	MSI Call
Seraseq® gDNA MSI-High Mix	TSO500	106	81	77.1	High

*MSI measurements are from replicate runs on the TSO500. MSI score is the ratio of the unstable MSI sites to the total number of sites detected (expressed as a percentage). The value must be >20% for an MSI-High result.

FEATURES AND BENEFITS

- Cell line or plasmid-based MSI reference material mix for analysis in molecular assays or NGS
- Offered as tumor-only (MSI-High) or tumor-normal (AF5% and AF20%) options
- Support MSI assay validation, LoD determination, and routine detection of MSI markers in cancer patient samples
- Variant AFs (AF5% and AF20% products) quantitated by ddPCR and qPCR/CE fragment length analysis assays
- Normal background DNA is a highly characterized GM24385 human genomic DNA known to be microsatellite stable (MSS)
- Manufactured within cGMP compliant and ISO 13485 certified facilities

ORDERING INFORMATION

Product Description	Kit Composition	Material No	Conc.	Volume	Total Mass (DNA)
Seraseq gDNA MSI-High Mix	gDNA - Tumor	0710-1670	20 ng/µl	25 µl	500 ng
Seraseq MSI Reference Panel Mix AF5%	gDNA – Tumor gDNA - Normal	0710-1675	2 x 20 ng/µl	2x 15 µl	2 x 300 ng
Seraseq MSI Reference Panel Mix AF20%	gDNA – Tumor gDNA - Normal	0710-1676	2 x 20 ng/µl	2x 15 µl	2 x 300 ng

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