

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	

¹There 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.

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- 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.