Seraseq®

Blood TMB Mix (Score 7 & 26) Products

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® Blood TMB Mix products are reference materials formulated for use with Next Generation Sequencing (NGS) assays that detect somatic mutations in human cancer patient samples. These products are intended for use as reference materials in the determination of the number of somatic mutations per genome 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

Material Number	Blood TMB Reference Materials
0710-2087	Seraseq® Blood TMB Mix Score 7
0710-2090	Seraseq® Blood TMB Mix Score 26

Each item consists of 3 vials at tumor fractions 0%, 0.5% and 2%; 3x10 ng/µl concentration; 3x20 µl fill volumes; and 3x200 ng total mass.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq Blood TMB Mix product as though it is capable of transmitting infectious agents. This product consists of purified and fragmented DNA from diseased (lung or breast cancer, i.e., tumor) and SNP-matched non-diseased (i.e., normal) human cell lines.

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 Blood TMB Mix product beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq Blood TMB Mix frozen at -20°C. After opening, record the date opened and the expiration date on the vial. Aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze-thaw cycles.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq Blood TMB Mix is formulated as a tumor-normal reference materials derived from expanded/cultured human cell line of diseased (tumor) and matching non-diseased (normal) patient, and 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

Each Seraseq Blood TMB Mix consist of 3 vials of fragmented (~188bp size, see Figure 1) and purified DNA from human cell lines (diseased and normal), blended at tumor fractions of 0% (WT), 0.5% and 2%. 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. Each vial of the Seraseq Blood TMB 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

Tissue TMB values were assigned using Whole Exome data for the matched parental tumor and normal DNA cell lines, which uses a VAF 5% cutoff for non-synonymous TMB variant detection. Applying the same criteria for Blood TMB is a challenge because at VAF 5% and 100% tumor, a 2% tumor fraction results in a VAF cutoff of 0.1%, which is below the claimed LoD of many cfDNA NGS assays.
https://www.q2labsolutions.com/en/genomics-laboratories/tso500-plasma

Blood TMB scores for each tumor fraction of the Seraseq Blood TMB Mix products were determined by a targeted NGS assay (TSO500 plasma) and analyzed by the associated bioinformatics pipeline. Results are shown in Table 1. Detection of somatic mutations may differ across different NGS panels, and concomitantly the Blood TMB scores determined by targeted NGS panels for the Seraseq Blood TMB Mix products may differ. The matched normal DNA that comprises ≥98% of total DNA in each product, contains some low frequency variants which should be treated as clonal hematopoiesis and subtracted from the Blood TMB Scores determined for the 2% and 0.5% blends. Each laboratory must establish an expected Blood TMB score for each of the Seraseq Blood TMB Mix products. 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. Additional support documents (VCFs of filtered mutations from analysis pipeline) are available by contacting us at CDx-info@LGCgroup.com

LIMITATIONS OF THE PROCEDURE

Seraseq Blood TMB 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 Blood TMB Mix is not a calibrator and should not be used for assay calibration. These materials are not whole-process controls and do not evaluate the methods used for specimen extraction. Adverse shipping and/or storage conditions or use of outdated product may produce erroneous results.

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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.

Figure 1: Example DNA fragment sizing in the Seraseq® Blood TMB Mix Score 7 for TF 0%, 0.5% and 2%.

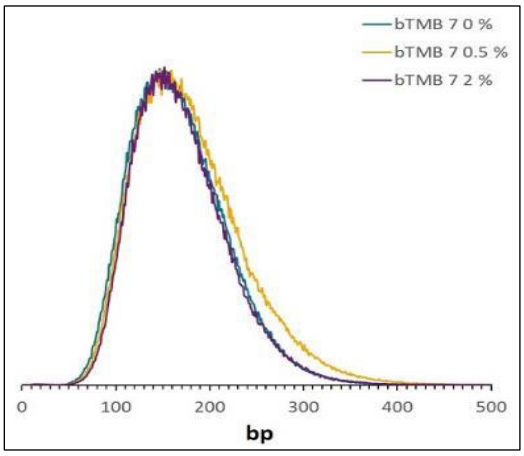

Table 1: Targeted NGS assay[1] determination of Blood TMB Scores for the Seraseq Blood TMB Mix products.

Blood TMB Reference Materials	Material Number	Blood TMB Score TF 0%[2]	Blood TMB Score TF 0.5%[2]	Blood TMB Score TF 2%[2]	Adjusted[3] Blood TMB Score (TF 0.5%)	Adjusted[3] Blood TMB Score (TF 2%)
Seraseq® Blood TMB Mix Score 7	0710-2087	7.5 ± 1.7	13.1 ± 2.6	17.9 ± 1.3	5.6 ± 2.1	10.4 ± 1.7
Seraseq® Blood TMB Mix Score 26	0710-2090	6.0 ± 0.5	20.7 ± 5.5	30.4 ± 1.8	14.7 ± 2.4	24.4 ± 1.5

[1]Analysis was performed using the ILMN TSO500 plasma assay, on a Novaseq 6000 NGS system.
[2]The average and one standard deviation from three technical replicate measurements are shown.
[3]Subtracts the TF 0% Blood TMB variant contributions (~6-7) for the tumor-derived (adjusted) scores.

Seraseq® gDNA, ctDNA and FFPE TMB Products

HUMAN CELL LINE DERIVED REFERENCE SAMPLES, TUMOR-NORMAL MATCHED SET, WITH RANGE OF TMB SCORES FOR VALIDATION AND USE OF TARGETED NGS PANEL IN TMB MEASUREMENTS.

HIGHLIGHTS

DERIVED FROM DISEASED HUMAN CELL LINES AND MATCHED NORMAL CELL LINES; IN PURIFIED DNA AND FFPE FORMATS.

REFERENCE STANDARDS DEVELOPED IN PARTNERSHIP WITH INDUSTRY EXPERTS.

HIGH-QUALITY MANUFACTURED REFERENCE MATERIAL; GUARANTEES CONSISTENT GROUND TRUTH

INTRODUCTION

In immuno-oncology (I-O), the goal is to enable a patient's immune system to locate and eliminate cancerous cells. Checkpoint inhibitors (CPI), which have recently improved the prognosis for a significant number of cancer patients, work by blocking inhibitory molecules such as PD-L1 and CTLA-4 that may be allowing cancers to evade the adaptive immune system. The US FDA has approved several CPIs such as ipilimumab (Yervoy®, 2011; BMS), pembrolizumab (Keytruda®, 2014; Merck), and nivolumab (Opdivo®, 2014; BMS) primarily for melanoma treatment, but extended to cover treatment of patients with Non-Small Cell Lung Cancer (NSCLC) and other renal cancers. However, by taking the brakes off the immune system, the use of CPI is associated with a significant risk of developing autoimmune disease.

In order to maximize the safety and efficacy of checkpoint inhibitors, it is beneficial to identify patients who are likely to respond to their use. Since the adaptive immune system can detect changes to proteins, it is thought that the number of somatic mutations that lead to such changes may be correlated with efficacy. The metric of non-silent somatic mutations per megabase of coding DNA has been termed tumor mutational burden (TMB), which has shown some correlation to the efficacy of checkpoint inhibitors. Assessments of TMB are being added to many NGS assays to enable their use for patient selection in I-O clinical trials, and perhaps as companion diagnostics to these therapies. However, it is also known that TMB scores can differ significantly between assays and especially around levels that may be clinical decision points.

SERASEQ TMB REFERENCE MATERIALS – gDNA, ctDNA AND FFPE

Purified gDNA TMB reference materials were made from human diseased cell lines and their matched peripheral blood (normal) lymphoblastoid cells derived from the same patients. FFPE TMB reference materials were made from human diseased cell lines, blended to 30% tumor content, formalin treated and paraffin embedded into FFPE blocks, then cut into 10 µm sections. Blood TMB (ctDNA) reference materials were made from human diseased cell lines and their SNP-matched normal cell lines, bulk mix is fragmented and sized to typical cfDNA fragment sizes, purified and blended at 0%, 0.5% and 2% tumor fractions. Tissue TMB/Blood TMB scores were determined using whole exome sequencing (WES – tissue TMB) and a targeted NGS panel (Blood TMB), and analyzed by TMB analysis pipelines in a tumor-normal setting (WES) or tumor-only setting filtered with germline variants (targeted panel).

TMB Reference Material	Cell Line	gDNA TMB Scores	FFPE TMB Scores	Blood TMB Scores
Seraseq® TMB Score 7	Small cell lung cancer; carcinoma (stage E)	7.2 ± 0.2	7.2 ± 0.4	5.6 ± 2.1 (0.5% TF) 10.4 ± 1.7 (2% TF)
Seraseq® TMB Score 9	Lung adenocarcinoma (stage 1)	9.5 ± 0.4	7.5 ± 1.3	N/A
Seraseq® TMB Score 20	Non-small cell lung cancer; carcinoma	20.1 ± 0.2	18.6 ± 0.5	TBD
Seraseq® TMB Score 26	Lung adenocarcinoma (stage 4)	25.8 ± 0.5	22.8 ± 3.6	14.7 ± 2.4 (0.5% TF) 24.4 ± 1.5 (2% TF)
Seraseq® TMB Score 13	B-Lymphocyte	12.6 ± 0.02	12.1 ± 0.3	TBD

ABOUT LGC SERACARE

TRUSTED SUPPLIER TO THE DIAGNOSTIC TESTING INDUSTRY FOR OVER 30 YEARS

HIGH-QUALITY CONTROL PRODUCTS, RAW BIOLOGICAL MATERIALS, AND IMMUNOASSAY REAGENTS

INNOVATIVE TOOLS AND TECHNOLOGIES TO PROVIDE ASSURANCE IN DIAGNOSTIC ASSAY PERFORMANCE AND TEST RESULTS

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: WWW.SERACARE.COM

FEATURES AND BENEFITS

- gDNA TMB:
  - 100% tumor-normal matched reference materials
  - Purified DNA in buffer
  - Ready as input into WES or targeted NGS library preparation
- FFPE TMB:
  - 30% tumor FFPE reference standards
  - FFPE sectioned to 10 µm per vial
  - Compatible with a range of FFPE extraction kits
- Blood TMB:
  - 0%, 0.5%, 2% tumor fractions to mimic tumor levels in cell free DNA
  - Purified ctDNA in buffer
  - Ready as input into targeted NGS library preparation
- TMB scores range of 7 to 26
- Manufactured in GMP-compliant and ISO 13485 certified facilities

ORDERING INFORMATION

Each part code is available for individual purchase.

Product	Format	Material No.	Concentration	Fill Volume	Total Mass
Seraseq® gDNA TMB Mix Score 7	100% tumor-normal matched set - provided in separate vials. No extraction required. Purified DNA in buffer.	0710-1326	50 ng/µL/vial (x2)	10 µl/vial (x2)	500 ng (x2)
Seraseq® gDNA TMB Mix Score 9		0710-1325	50 ng/µL/vial (x2)	10 µl/vial (x2)	500 ng (x2)
Seraseq® gDNA TMB Mix Score 13		0710-1586	50 ng/µL/vial (x2)	10 µl/vial (x2)	500 ng (x2)
Seraseq® gDNA TMB Mix Score 20		0710-1324	50 ng/µL/vial (x2)	10 µl/vial (x2)	500 ng (x2)
Seraseq® gDNA TMB Mix Score 26		0710-1323	50 ng/µL/vial (x2)	10 µl/vial (x2)	500 ng (x2)
Seraseq® Blood TMB Mix Score 7	Tumor-normal blends at 0%, 0.5% & 2% - provided in separate vials. No extraction required. Purified DNA in buffer.	0710-2087	50 ng/µL/vial (x3)	20 ul/vial (x3)	200 ng (x3)
Seraseq® Blood TMB Mix Score 26		0710-2090	50 ng/µL/vial (x3)	20 ul/vial (x3)	200 ng (x3)
Seraseq® FFPE TMB RM Score 7	30% tumor content. Full Process. Extraction Required - FFPE	0710-1310	1 FFPE curl/vial (x2)	10 µm (x2)	>100 ng/curl* (x2)
Seraseq® FFPE TMB RM Score 9		0710-1308	1 FFPE curl/vial (x2)	10 µm (x2)	>100 ng/curl* (x2)
Seraseq® FFPE TMB RM Score 13		0710-1618	1 FFPE curl/vial (x2)	10 µm (x2)	>100 ng/curl* (x2)
Seraseq® FFPE TMB RM Score 20		0710-1309	1 FFPE curl/vial (x2)	10 µm (x2)	>100 ng/curl* (x2)
Seraseq® FFPE TMB RM Score 26		0710-1307	1 FFPE curl/vial (x2)	10 µm (x2)	>100 ng/curl* (x2)

**Based on Qiagen QIAamp DNA FFPE Tissue Kit and the Qubit dsDNA HS Assay*

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MKT-00504-03

Seraseq® Blood TMB Mix Score 7, 13, 20, 26

0710-2087 | 0710-2088 | 0710-2089 | 0710-2090

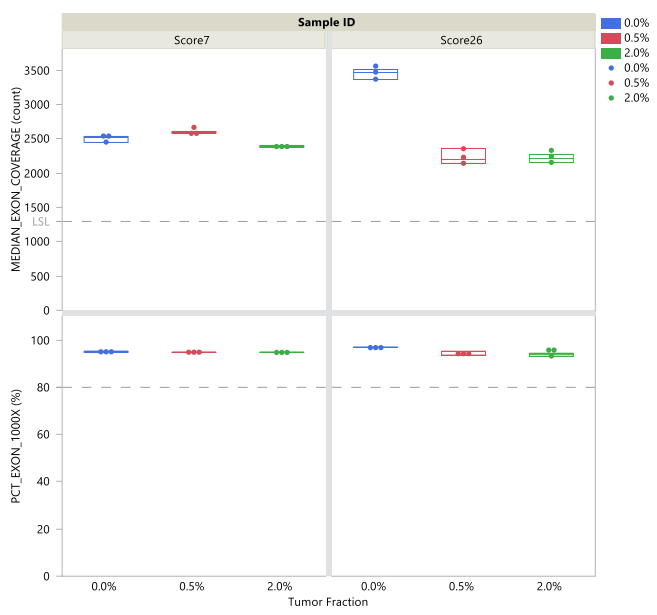
This Product Datasheet provides more details around the Blood TMB assessments for Seraseq Blood TMB Mix (For score 7 and 26 only) determined using the TruSight Oncology 500 ctDNA panel.

Blood TMB Materials:

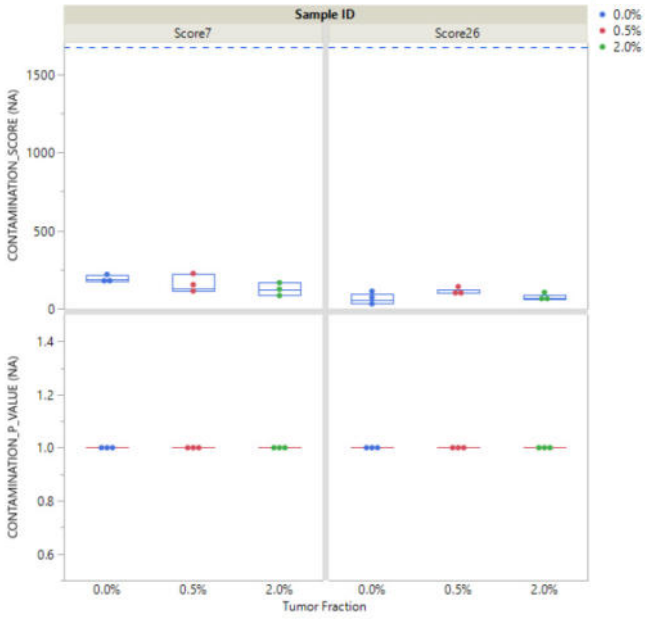
- Human diseased cell-lines and their SNP-matched normal cell lines
- Fragmented to ctDNA sizes of ~165 bp
- Blended at 3 tumor fractions – 0%, 0.5% and 2%
- Blood TMB Scores generated by a targeted NGS panel

Blood TMB Measurements:

TMB measurements of the Seraseq Blood TMB Mix Score 7 and 26 were performed using the TruSight Oncology 500 ctDNA assay. Each tumor fraction sample was analyzed in triplicate. All samples passed sequencing library QC metrics (see, e.g., Figure 1).



(a)



(b)

FIGURE 1: Sequencing library QC metrics check for (a) Minimum Exon Coverage (MEC; >1000X) and (b) Contamination analysis for all samples and replicates of the Seraseq Blood TMB products. All passed QC.

Blood TMB Score Analysis

- Assay = TruSight Oncology 500 ctDNA
- System = NovaSeq 6000 / S2 Flowcell
- Variant Call Min Depth = 1000X
- VAF > 0.2%
- Coverage Uniformity = 80% of exons covered at 1000X
- Blood TMB Analysis = DRAGEN TruSight Oncology 500 ctDNA Analysis Software v1.1

Sample	%Tumor Fraction	Conc (ng/µl)* Chromosome	Input (ng)	Reps	Ave Blood TMB Score**	Adjusted Blood TMB Scores***
Seraseq Blood TMB Mix Score 7	0%	41	30	3	7.5 ± 1.7	0
	0.5%	57.6	30	3	13.1 ± 2.6	5.6 ± 2.1
	2%	41.2	30	3	17.9 ± 1.3	10.4 ± 1.7
Seraseq Blood TMB Mix Score 26	0%	64.8	30	3	6.0 ± 0.5	0
	0.5%	11	30	3	20.7 ± 5.5	14.7 ± 2.4
	2%	11.9	30	3	30.4 ± 1.8	24.4 ± 1.5

*Qubit dsDNA.
**Total bTMB score (syn + non-syn).
*** Score subtracts bTMB variants of 0% tumor (WT) blend.

The observed Blood TMB scores for the 2% and 0.5% mix represent total TMB for synonymous and non-synonymous variants derived from the cancer cell line + the variants harbored in the 0% cell line used as the background. Hence, the Blood TMB scores in the 2% and 0.5% mixes are elevated by ~6-7 Mut/Mb observed in the 0% mix.

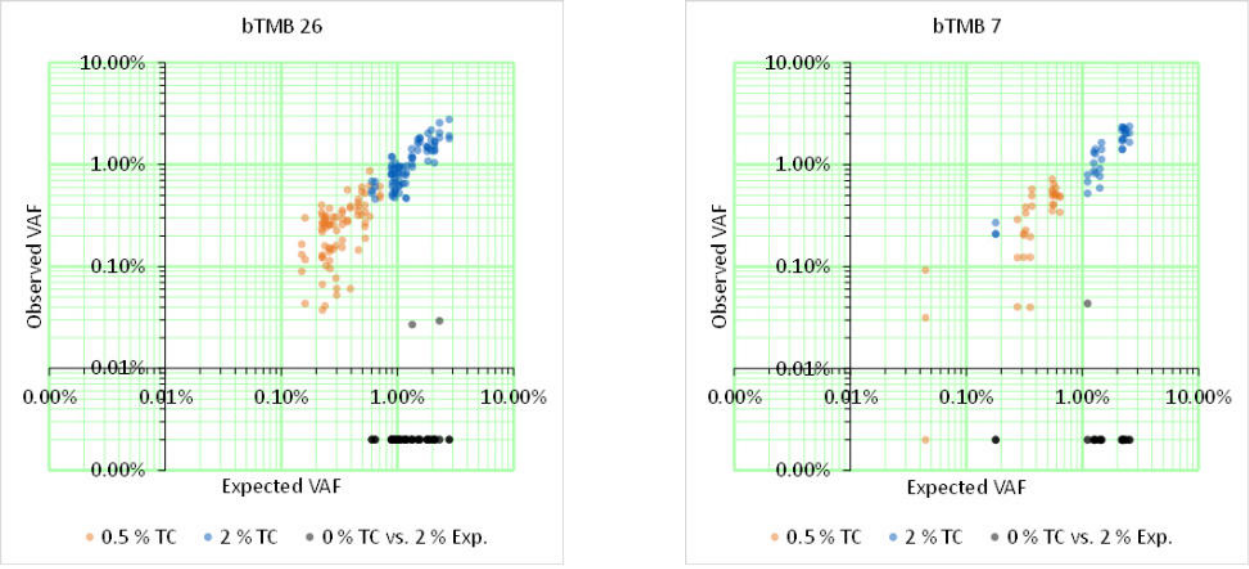


FIGURE 2: Analysis of observed versus expected VAFs for the bTMB variants for all replicates. Black dots (•) compare the 0 % samples to what would be expected for 2 %. Blue dots (•) compare observed 2 % to expected 2 %. Orange dots (•) compare observed 0.5 % to expected 0.5 %. Noise (when present) seems to be around 0.03 % for the bTMB variants.

TMB Score comparisons: Tissue-derived vs Blood TMB

TMB Reference Material	Cell Line	gDNA TMB Scores	FFPE TMB Scores	Blood TMB Scores
Seraseq TMB Score 7	Small cell lung cancer; carcinoma	7.2 ± 0.2	7.2 ± 0.4	5.6 ± 2.1 (0.5% TF) 10.4 ± 1.7 (2% TF)
Seraseq TMB Score 9	Lung adenocarcinoma	9.5 ± 0.4	7.5 ± 1.3	N/A
Seraseq TMB Score 13	B-Lymphocyte	12.6 ± 0.02	12.1 ± 0.3	Pending
Seraseq TMB Score 20	Non-small cell lung cancer; carcinoma	20.1 ± 0.2	18.6 ± 0.5	Pending
Seraseq TMB Score 26	Lung adenocarcinoma	25.8 ± 0.5	22.8 ± 3.6	14.7 ± 2.4 (0.5% TF) 24.4 ± 1.5 (2% TF)

TMB variant lists are available by contacting us at CDx-info@LGCgroup.com.

CONTACT US

For additional information on these TMB reference standards, please visit our dedicated product page at https://www.seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Immuno-oncology/, or call **+1-800-676-1881.**

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MKT-00615-01

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