Seraseq®

gDNA BRCA1/2 LGR Somatic Mutation Mix

Package Insert

NAME AND INTENDED USE

The Seraseq® gDNA BRCA1/2 LGR Somatic Mutation Mix is a reference material intended for use in the development, validation, and evaluation of routine performance of Next Generation Sequencing (NGS) (and other amplified nucleic acid-based methods) that identify somatic and inherited (germline) variants in the genes *BRCA1* and *BRCA2* associated with breast and ovarian cancers. This reference material is suitable for use by clinical laboratories, research institutions, and diagnostic assay developers to ensure consistent and reliable results across different sequencing runs and laboratory conditions. The Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix is not intended for use in patient diagnosis, treatment, or in any therapeutic procedures. It is intended for use by trained laboratory personnel proficient in NGS technologies and familiar with proper laboratory practices and quality control procedures.
For Research Use Only (RUO). Not for use in diagnostic procedures.

REAGENT PROVIDED

Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix is a mixture of synthetic DNA constructs and genomic DNA extracted from the human cell line GM24385. It contains 20 synthetic mutations in the BRCA1 and BRCA2 genes (not including those present in the GM24385 background) (Table 2). The product is formulated to simulate a tumor state for each mutation at a 10% variant allele frequency (VAF) confirmed by droplet digital PCR and measured by NGS.

Table 1. Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix

Material No.	Product	Format
0730-0568	Seraseq® gDNA BRCA1/2 LGR Somatic Mutation Mix	1x 25 µL

One (1) vial, 25 µL per vial, 375 ng total mass, at a nominal concentration of 15 ng/µL is provided. The product is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer. Refer to the batch-specific Technical Product Report for exact concentration and VAF measured. Manufactured in the USA.

WARNINGS AND PRECAUTIONS

Safety and Handling Precautions

Handle Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. 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. Avoid contamination of the product when opening and closing the vials. Dispose of all specimens and materials appropriately.

STORAGE INSTRUCTIONS

Store Seraseq gDNA BRCA1/2 LGR Somatic 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 gDNA BRCA1/2 LGR Somatic Mutation Mix 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 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 gDNA BRCA1/2 LGR Somatic Mutation Mix should be integrated into library preparation after the DNA isolation step; no further purification or DNA isolation is needed. If a DNA shearing step is part of the workflow, the reference material should be sheared and go through the target selection and library preparation in parallel with test specimens. Refer to standard assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

Table 2 indicates each of the mutations represented in the Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix. While the presence and frequency of each variant in this product was confirmed during manufacture using digital PCR assays and NGS, there may be differences in observed allele frequencies due to assay characteristics. The product does not have assigned values for allele frequencies of the variants present. Furthermore, specific detection of variants and variant allele frequencies within the product will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory must establish an assay-specific expected value and acceptance range for each variant and lot of the Mutation Mix prior to its routine use. 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 gDNA BRCA1/2 LGR Somatic Mutation Mix MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix is not compatible with MLPA (Multiplex ligation-dependent probe amplification) assays and NGS analysis methods based only on coverage depth, since the large genomic rearrangements do not reflect copy losses or gains across the whole DNA sequence.
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 gDNA BRCA1/2 LGR Somatic 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. The product 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.

REFERENCE

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.

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Table 2. List of Mutations

Gene ID	Mutation Type	Nucleotide Change	Amino acid change	GRCh37 Location	GRCh38 Location	Transcript	Variant Length
BRCA1	Deletion	c.4487_4675+2del	Splice variant	17:41226346_41226536	17:43074329_43074519	NM_007294.4	191
	Insertion	c.4186_4357dup	p.R1397Yfs*2	17:41234421_41234592	17:43082404_43082575	NM_007294.4	172
	Deletion	c.2071_2171del	p.R691*	17:41245377_41245477	17:43093360_43093460	NM_007294.4	101
	Deletion	c.4987_5074del	p.V1665Sfs*8	17:41219625_41219712	17:43067608_43067695	NM_007294.4	88
	Deletion	c.5279_5332del	p.I1760_D1778delinsN	17: 41203080_41203133	17: 43051063_43051116	NM_007294.4	54
	Indel	c.5209_5248delinsTC	p.R1737Sfs*80	17:41209098_41209137	17:43057081_43057120	NM_007294.4	40
	Indel	c.2820_2830delinsAA GATAAGCCAGTTTGATAA	p.D940_C944delinsER*	17:41244718_41244728	17:43092701_43092711	NM_007294.4	11
	Deletion	c.1961del	p.K654Sfs*47	17:41245587	17:43093570	NM_007294.4	1
	SNV	c.4327C>T	p.R1443*	17:41234451	17:43082434	NM_007294.4	1
	SNV	c.441+2T>G	Splice variant	17:41256137	17:43104120	NM_007294.4	1
BRCA2	Deletion	c.8757-2_9023del	Splice variant	13:32953452_32953956	13:32379315_32379819	NM_000059.4	505
	Deletion	c.2886_3144del	p.H962Qfs*6	13:32911378_32911636	13:32337241_32337499	NM_000059.4	259
	Deletion	c.68_316del	p.D23_L105del	13:32893214_32893462	13:32319077_32319322	NM_000059.4	249
	Indel	c.5150_5226delinsTACTTAATA CTTATTAAGTATTA	p.E1717_N1742delinsVLNTY*	13:32913642_32913718	13:32339505_32339581	NM_000059.4	77
	Indel	c.891_899delinsGATACTTCAG	p.T298Ifs*7	13:32906506_32906514	13:32332369_32332377	NM_000059.4	9
	Deletion	c.5436del	p.E1812Dfs*3	13:32913927	13:32339790	NM_000059.4	1
	SNV	c.8167G>C	p.D2723H	13:32937506	13:32363369	NM_000059.4	1
	SNV	c.8331+2T>A	Splice variant	13:32937672	13:32363535	NM_000059.4	1
	SNV	c.910G>T	p.E304*	13:32906525	13:32332388	NM_000059.4	1
	Insertion	c.2407dup	p.Y803Lfs*2	13:32910898	13:32336761	NM_000059.4	1

NOTE: Above list does not include variants present in the GM24385 background. Substitution refers to a single nucleotide variant; Indel is defined as a deletion/insertion less than 10 base pairs, and large genomic rearrangements (LGRs) (deletions or insertions) are defined as longer than 10 base pairs. Target variant allele frequency (VAF) at 10%.