

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® FFPE BRCA1/2 LGR (Large Genomic Rearrangement) Reference Material contains 20 variants across the BRCA1 and BRCA2 genes in the genomic background of GM24385 (Table 2). The product is supplied as one 10 µm Formalin-Fixed Paraffin Embedded (FFPE) curl supplied in one vial.

Seraseq FFPE BRCA1/2 LGR Reference Material is intended for use with Next Generation Sequencing (NGS) assays or amplified nucleic acid-based methods that identify somatic and inherited (germline) variants in the genes *BRCA1* and *BRCA2* under a given set of bioinformatics pipeline parameters. This product is intended for use as a full process quality reference material in the development, validation, and evaluation of routine performance of laboratory tests.

REAGENTS

Table 1. Seraseq® FFPE BRCA1/2 LGR Reference Material

| Material No. | Product | Unit |
|--------------|--|--------|
| 0730-0564 | Seraseq® FFPE BRCA1/2 LGR Reference Material | 1 curl |

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

CAUTION: Handle Seraseq FFPE BRCA1/2 LGR Reference Material as though it is capable of transmitting infectious agents. This product is formulated using an engineered human cell line derived from GM24385, which is a B-lymphocytic, male cell line from the Genome in a Bottle (GIAB) Project/ the Personal Genome Project offered by the NIGMS Human Genetic Cell Repository (<https://catalog.coriell.org/1/NIGMS>).

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 FFPE BRCA1/2 LGR Reference Material at 2-8°C. Do not use the product beyond the expiration date.

PROCEDURE

Materials Provided

Seraseq FFPE BRCA1/2 LGR Reference Material consists of engineered cells which have been formalin treated and embedded in paraffin to create an FFPE block, which is then sectioned into 10 µm curls. One 10 µm FFPE curl is provided per vial.

Materials Required but not Provided

Seraseq FFPE BRCA1/2 LGR Reference Material requires DNA extraction. Refer to instructions supplied by manufacturers of the DNA extraction kit to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Seraseq FFPE BRCA1/2 LGR Reference Material must go through a DNA extraction process. Refer to your assay procedures in order to determine the amount of extracted material to use in library preparation.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

Seraseq FFPE BRCA1/2 LGR Reference Material is compatible with nucleic acid extraction methods used for FFPE specimens. Minimum DNA extraction yields per FFPE curl (10 µm) when using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Cat.# 56404) and quantitated by ThermoFisher's Qubit dsDNA HS assay is 100 ng.

While the presence and frequency of each variant in this product was confirmed during manufacture using digital PCR assays and / or functional NGS, there may be differences in observed allele frequencies due to assay characteristics. The Seraseq FFPE BRCA1/2 LGR Reference Material does not have assigned values for allele frequencies of the variants present. Furthermore, specific detection of variants and variant allele frequencies within Seraseq FFPE BRCA1/2 LGR Reference Material 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 reference material 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 FFPE BRCA1/2 LGR REFERENCE MATERIAL 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 instructions may produce unreliable results. LGC Clinical Diagnostics does not claim that others can duplicate test results exactly. Seraseq FFPE BRCA1/2 LGR Reference Material 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.

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 2: List of 20 DNA variants in Seraseq® FFPE BRCA1/2 LGR Reference Material

| Gene ID | Variant Type | Nucleotide change | Protein change | GRCh37 Location | GRCh38 Location | dbSNP ID | Variant Length (bp) |
|---------|--------------|--|---------------------------|-----------------|-----------------|--------------|---------------------|
| BRCA1 | Deletion | c.1961del | p.K654Sfs*47 | 41245586 | 43093569 | rs80357522 | 1 |
| BRCA1 | Deletion | c.4487_4675+2del | Splice variant | 41226345 | 43074328 | rs1555581778 | 191 |
| BRCA1 | Insertion | c.4186_4357dup | p.R1397Yfs*2 | 41234420 | 43082403 | N/A | 172 |
| BRCA1 | Deletion | c.2071_2171del | p.R691* | 41245376 | 43093359 | rs1555590294 | 101 |
| BRCA1 | Deletion | c.4987_5074del | p.V1665Sfs*8 | 41219624 | 43067607 | N/A | 88 |
| BRCA1 | Deletion | c.5279_5332del | p.I1760_D1778delinsN | 41203079 | 43051062 | rs1555575677 | 54 |
| BRCA1 | INDEL | c.5209_5248delinsTC | p.R1737Sfs*80 | 41209098 | 43057081 | rs273901753 | 40 |
| BRCA1 | INDEL | c.2820_2830delinsAAGATAAGCCAGTTTGATAA | p.D940_C944delinsER* | 41244717 | 43092700 | rs1555588883 | 11 |
| BRCA1 | SNV | c.4327C>T | p.R1443* | 41234451 | 43082434 | rs41293455 | 1 |
| BRCA1 | SNV | c.441+2T>G | Splice variant | 41256137 | 43104120 | rs397509173 | 1 |
| BRCA2 | Insertion | c.2407dup | p.Y803Lfs*2 | 32910899 | 32336762 | rs2072457050 | 1 |
| BRCA2 | Deletion | c.2886_3144del | p.H962Qfs*6 | 32911376 | 32337239 | rs2072467220 | 259 |
| BRCA2 | Deletion | c.8755-2_9023del | Splice variant | 32953451 | 32379314 | N/A | 505 |
| BRCA2 | Deletion | c.68_316del | p.D23_L105del | 32893210 | 32319073 | N/A | 249 |
| BRCA2 | INDEL | c.5150_5226delinsTACTTAATACTTATTAAGTATTA | p.E1717_N1742delinsVLNTY* | 32913641 | 32339504 | N/A | 77 |
| BRCA2 | INDEL | c.891_899delinsGATACTTCAG | p.T298Ifs*7 | 32906505 | 32332368 | rs276174914 | 9 |
| BRCA2 | Deletion | c.5436del | p.E1812Dfs*3 | 32913926 | 32339789 | rs397507351 | 1 |
| BRCA2 | SNV | c.8167G>C | p.D2723H | 32937506 | 32363369 | rs41293511 | 1 |
| BRCA2 | SNV | c.8331+2T>A | Splice variant | 32937672 | 32363535 | rs398122602 | 1 |
| BRCA2 | SNV | c.910G>T | p.E304* | 32906525 | 32332388 | rs1593891461 | 1 |

NOTE: Above list does not include variants present in the GM24385 background.

Substitution refers to single nucleotide variant; Indels are defined as deletion/insertions less than 10 base pairs, and LGRs (deletions or insertions) are larger than 10 base pairs.

The annotations of nucleotide and protein changes refer to transcripts NM_007294.4 (BRCA1) and NM_000059.4 (BRCA2).