

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® SARS-CoV-2 Mutation RNA Mix product is a reference material formulated for use with Next Generation Sequencing (NGS) assays that detect variant mutations in SARS-CoV-2 positive patient samples. This product is intended for use as a reference material in the development and validation of routine laboratory tests used to detect SARS-CoV-2 disease gene variants 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	Product Name
0740-0075	Seraseq® SARS-CoV-2 Mutation RNA Mix

Product consists of 3 members representing 3 different variant allele frequencies: 1%, 5%, and 20%. Each member contains SARS-CoV-2 mutant RNA mixed with the background of the Wuhan SARS-CoV-2 strain RNA. Each member also contains human total RNA from the GM24385 cell line. Each vial contains a 25 µL fill volume at a total RNA concentration of 25 ng/µL and a SARS-CoV-2 RNA concentration of 5.0E+06 copies/mL.

WARNINGS AND PRECAUTIONS

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

CAUTION: Handle Seraseq SARS-CoV-2 Mutation RNA Mix product as though it is capable of transmitting infectious agents. This product consists of purified RNA from biosynthetic constructs blended into a background GM24385 WT cell line.

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 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 SARS-CoV-2 Mutation RNA Mix product beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq SARS-CoV-2 Mutation RNA Mix frozen at -70°C. Shelf life when stored under these conditions is 2 years from date of manufacture.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq SARS-CoV-2 Mutation RNA Mix is a mixture of human total RNA purified from GM24385 cell line, wild-type Wuhan SARS-CoV-2 RNA and SARS-CoV-2 variant RNA constructs. It 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**

Seraseq SARS-CoV-2 Mutation RNA Mix consists of RNA purified from a reference cell line, GM24385, plus constructs containing SARS-CoV-2 variant RNA mixed at defined allele frequencies within a background of wild type Wuhan SARS-CoV-2 strain RNA. The purified RNA 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 RNA isolation. No further purification or RNA 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 SARS-CoV-2 Mutation RNA 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 indicates each of the mutations represented in the Seraseq SARS-CoV-2 Mutation RNA Mix. Detection of mutations may differ across different NGS panels and different test reagent lots. While the presence and frequency of each mutation in this product was confirmed during manufacture using functional NGS and/or digital PCR assays, there may be differences in observed allele frequencies due to assay characteristics. Seraseq SARS-CoV-2 Mutation RNA Mix does not have assigned values for allele frequencies of the mutations present in the product. Each laboratory must establish an assay-specific expected value for each mutation and each lot of Seraseq SARS-CoV-2 Mutation RNA Mix. 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 are available by contacting us at CDx.Marketing@LGCgroup.com

LIMITATIONS OF THE PROCEDURE

Seraseq SARS-CoV-2 Mutation RNA 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. LGC Clinical Diagnostics does not claim that others can duplicate test results exactly. Seraseq SARS-CoV-2 Mutation RNA 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.

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: List of 82 SARS-CoV-2 mutations in the Seraseq SARS-CoV-2 Mutation RNA Mix product.

Amino Acid Change	Nucleotide Change	Gene	Viral isolate
T85I	1059C>T	Nsp2	B.1.351
S370L	3828C>T	Nsp3	P1
K837N	5230G>T	Nsp3	B.1.351
A890D	5388C>A	Nsp3	B.1.1.7
K977Q	5648A>C	Nsp3	P1
I1683T	7767T>C	NSP3	B.1.258
K90R	10323A>G	Nsp5	B.1.351
S106-108 del	11288-11296del	Nsp6	B.1.525
I65V	12878A>G	NSP9	B.1.429
P323L	14408C>T	Nsp12	B.1.351
E341D	17259G>T	Nsp13	global
A185S	13993G>T	NSP12	B.1.160
V776L	15766G>T	NSP12	B.1.160
K218R	16889A>G	NSP13	B.1.160
D260Y	17014G>T	NSP13	B.1.429
E261D	17019G>T	NSP13	B.1.160
S13I	21600G>T	Spike	B.1.429
L18F	21614C>T	Spike	P1; B.1.351
T20N	21621C>A	Spike	P1
P26S	21638C>T	Spike	P1
Q52R	21717A>G	Spike	B.1.525 & B1.1.207
H69del V70del	21765-21770del	Spike	B.1.1.7
D80A	21801A>C	Spike	B.1.351
D138Y	21974G>T	Spike	P1
G142V	21987G>T	Spike	B.1.1.7
Y145del	21993-21995del	Spike	B.1.1.7
W152C	22018G>T	Spike	B.1.429
R190S	22132G>T	Spike	P1
D215G	A22206A>G	Spike	B.1.351
L242del A243del L244del	22286-22294del	Spike	B.1.351
K417N	22813G>T	Spike	B.1.351
N439K	22879C>A	Spike	B.1.222
L452R	22917T>G	Spike	B.1.429
Y453F	22920A>T	Spike	B.1.1.7
E484K	23012G>A	Spike	B.1.351; P1
N501Y	23063A>T	Spike	B.1.1.7; B.1.351; P1
A570D	23271C>A	Spike	B.1.1.7
D614G	23403A>G	Spike	B.1.1.7; B.1.351; P1

Amino Acid Change	Nucleotide Change	Gene	Viral isolate
H655Y	23525C>T	Spike	P1
Q677H	23593G>C	Spike	B.1.525 & B1.1.207
P681H	23604C>A	Spike	B.1.1.7
I692V	23636A>G	Spike	Cluster 5 DK
A701V	23664C>T	Spike	B.1.351
T716I	23709C>T	Spike	B.1.1.7
F888L	24224T>C	Spike	B.1.525 & B1.1.207
L938F	24374C>T	Spike	B.1.429
S982A	24506T>G	Spike	B.1.1.7
T1027I or Y	24642C>T	Spike	P1
D1118H	24914G>C	Spike	B.1.1.7
S1147L	25002C>T	Spike	Cluster 5
V1176F	25088G>T	Spike	P1
K1191N	25135G>T	Spike	B.1.429
M1229I	25249G>T	Spike	Cluster 5 DK
Q57H	25563G>T	ORF3a	B.1.351
S171L	25904C>T	ORF3a	B.1.351
S253P	26149T>C	ORF3a	P1
L21F	26305C>T	E	B.1.525
P71L	26456C>T	E	B.1.351
I82T	26767T>C	M	B.1.525
K162N	27008G>T	M	B.1.351
V93F	27670G>T	ORF7a	B.1.351, B.1.160
Q27*	27972C>T	ORF8	B.1.1.7
R52I	28048G>T	ORF8	B.1.1.7
Y73C	28111A>G	ORF8	B.1.1.7
E92K	28167G>A	ORF8	P1
Silent	28263insAACA	intergenic/non CDS	P1
D3L	28280_2delinsCTA	N	B.1.1.7
A12G	28308C>G	N	B.1.525
P80R	28512C>G	N	P1
Silent	28877A>T	N	P1
Silent	28878G>C	N	P1
G204R R203K	28881GGG>AAC	N	P1 & B.1.1.7
T205I	28887C>T	N	B.1.351
S235F	28975C>T	N	B.1.1.7
M234I	28975G>C	N	multiple
Q289H	29140G>T	N	B.1.243
Silent	29200C>T	N	Unclear
A376T	29399G>A	N	B.1.160