

**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™ Fusion RNA Mix v3 is formulated for use with targeted Next Generation Sequencing (NGS) assays that detect RNA expressed from gene fusions common in cancer. This product is intended as a quality reference material for translational and disease research testing to monitor library preparation, sequencing, and fusion RNA detection under a given set of bioinformatics pipeline parameters. *For Research Use Only. Not for use in diagnostic procedures.*

**SUMMARY**

A well-designed quality control program provides added confidence in the reliability of results obtained for unknown specimens. The use of independent reference materials may provide valuable information concerning assay sensitivity and bioinformatics pipeline analysis.

**PRINCIPLES OF THE PROCEDURE**

Seraseq Fusion RNA Mix v3 is ready to use in NGS assays in steps that follow RNA isolation. No further purification or RNA isolation is needed.

**REAGENTS**

Item No. 0710-0431. 1 vial, 25 µL per vial, 25 ng/µL concentration

**WARNINGS AND PRECAUTIONS**

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

CAUTION: Handle Seraseq Fusion RNA Mix v3 as though it is capable of transmitting infectious agents. This product is formulated using total RNA from human cell line GM24385, which is a B-lymphocytic, male cell line from 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 specimens<sup>1</sup>. 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 Fusion RNA Mix v3 beyond the expiration date. Avoid contamination of the product when opening and closing the vial. Limit the number of freeze thaws this product is exposed to by creating single-use aliquots, if necessary.

**STORAGE INSTRUCTIONS**

Store Seraseq Fusion RNA Mix v3 at -70 °C. Shelf life when stored under these conditions is two years from date of manufacture.

**INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION**

Seraseq Fusion RNA Mix v3 is a mixture of human total RNA purified from GM24385 cell line and biosynthetic RNA. It should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and the vial should be discarded.

**PROCEDURE**

**Materials Provided**

Seraseq Fusion RNA Mix v3 consists of total cellular RNA purified from GM24385 cell line and biosynthetic RNA. The RNA is in 1 mM Tris, pH 8.0, aqueous buffer. 25 µL is provided per vial and the concentration is 25 ng/µL.

**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 Fusion RNA Mix v3 may be input directly into a reverse transcription assay step in parallel with the test specimens prior to target selection and library preparation. Refer to your usual assay procedures in order to determine the amount of material to use.

**Quality Control**

Seraseq Fusion RNA Mix v3 does not have assigned values for the proportion of fusion transcripts relative to wild-type transcripts for the same genes, or the overall quantity of fusion transcripts. However, the product is tested using fusion-specific digital PCR quantitation to determine approximate transcript level for each fusion RNA listed in Table 1. There are many reasons why fusions contained in the product may not be positively detected, which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of each lot of Seraseq Fusion RNA Mix v3 with each assay system prior to its routine use.

**EXPECTED RESULTS & INTERPRETATION OF RESULTS**

Detection of fusion RNA and exon skipping events may differ across different NGS fusion RNA panels and different test reagent lots. While each fusion RNA is present at a similar level as determined by fusion specific digital PCR-based assays, and functional NGS-based assays confirm the presence of all 16 fusion RNA variants, there may be apparent differences in observed fusion levels due to assay characteristics. The fusion RNA species in this product are NOT present at the DNA level. Each laboratory must establish an assay-specific expected value for each fusion and each lot of Seraseq Fusion RNA Mix v3. 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. Additional support documents are available online at [www.seracare.com/oncology](http://www.seracare.com/oncology).

Table 1 indicates each of the fusion genes represented.

#### LIMITATIONS OF THE PROCEDURE

Seraseq Fusion RNA Mix v3 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 Fusion RNA Mix v3 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 storage conditions or use of outdated product may produce erroneous results.

#### SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Fusion RNA Mix v3 has been designed for use with targeted NGS fusion RNA panels for the purposes of assessing assay characteristics. The product is manufactured from purified human total RNA as well as biosynthetic RNA. Procedures for implementing a quality assurance program and monitoring test performance on a routine basis must be established by each individual laboratory.

#### 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: Fusion RNA variants and exon skipping events in the Seraseq Fusion RNA Mix v3**

	RNA Fusion	5' Partner	3' Partner	HGVS Name
1	EML4-ALK	EML4 exon 13	ALK exon 20	EML4{NM_019063.4}:r.1_1763_ALK{NM_004304.4}:r.4125_6265
2	KIF5B-RET	KIF5B exon 24	RET exon 11	KIF5B{NM_004521.2}:r.1_3231_RET{NM_020975.4}:r.2070_5617
3	NCOA4-RET	NCOA4 exon 8	RET exon 12	NCOA4{NM_001145260.1}:r.1_1014_RET{NM_020975.4}:r.2327_5617
4	CD74-ROS1	CD74 exon 6	ROS1 exon 34	CD74{NM_001025159.2}:r.1_812_ROS1{NM_002944.2}:r.5757_7368
5	SLC34A2-ROS1	SLC34A2 exon 4	ROS1 exon 34	SLC34A2{NM_006424.2}:r.1_460_ROS1{NM_002944.2}:r.5757_7368
6	TPM3-NTRK1	TPM3 exon 7	NTRK1 exon 9	TPM3{NM_153649.3}:r.1_794_NTRK1{NM_001012331.1}:r.1234_2637
7	FGFR3-BAIAP2L1	FGFR3 exon 17	BAIAP2L1 exon 2	FGFR3{NM_001163213.1}:r.1_2530_BAIAP2L1{NM_018842.4}:r.315_3682
8	PAX8-PPARG1	PAX8 exon 9	PPARG1 exon 3	PAX8{NM_003466.3}:r.1_1253_PPARG{NM_138712.3}:r.246_1892
9	FGFR3-TACC3	FGFR3 exon 17	TACC3 exon 11	FGFR3{NM_000142.4}:r.1_2530_TACC3{NM_006342.2}:r.2097_2847
10	ETV6-NTRK3	ETV6 exon 5	NTRK3 exon 15	ETV6{NM_001987.4}:r.1_1283_NTRK3{NM_001012338.2}:r.1892_3004
11	LMNA-NTRK1	LMNA exon 2	NTRK1 exon 10	LMNA{NM_170707.3}:r.1_762_NTRK1{NM_001012331.1}:r.1290_2647
12	SLC45A3-BRAF	SLC45A3 exon 1	BRAF exon 8	SLC45A3{NM_033102.2}:r.1_109_BRAF{NM_004333.4}:r.1042_2949
13	TMPRSS2-ERG	TMPRSS2 exon 1 (5' UTR)	ERG exon 2	TMPRSS2{NM_005656.3}:r.1_78_ERG{NM_004449.4}:r.124_5042
14	EGFR-SEPT14	EGFR exon 24	SEPT14 exon 10	EGFR{NM_005228.3}:r.1_3192_SEPT14{NM_207366.2}:r.1237_3785
15	MET exon 14 skipping	MET exon 13	MET exon 15	MET{NM_001127500.2}:r.3144_3284del
16	EGFR vIII	EGFR exon 1	EGFR exon 8	EGFR{NM_005228.3}:r.335_1135del