

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 v4 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 v4 is ready to use in NGS assays in steps that follow RNA isolation. No further purification or RNA isolation is needed.

REAGENTS

Item No. 0710-0497. 1 vial, 25 µL per vial, 25 ng/µL. See Certificate of Analysis for lot specific information.

WARNINGS AND PRECAUTIONS

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

CAUTION: Handle Seraseq Fusion RNA Mix v4 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 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 Fusion RNA Mix v4 beyond the expiration date. Avoid contamination of the product when opening and closing the vial. Based on data, product is stable for up to five (5) freeze thaw cycles. Limit the number of freeze thaws this product is exposed to by creating single-use aliquots in low nucleic acid binding vials, if necessary.

STORAGE INSTRUCTIONS

Store Seraseq Fusion RNA Mix v4 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 v4 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 v4 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. See Certificate of Analysis for lot specific information.

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 v4 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 v4 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 v4 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 18 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 v4. 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.

Table 1 Indicates each of the fusion RNA variants and exon skipping events

LIMITATIONS OF THE PROCEDURE

Seraseq Fusion RNA Mix v4 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 v4 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 v4 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 v4

RNA Fusion	5' Partner	3' Partner	HGVS Name	COSF
CCDC6-RET	CCDC6 ex 1	RET ex 12	CCDC6{NM_005436.5}:r.1_435_RET{NM_020975.6}:r.2327_5617	COSF1272
CD74-ROS1	CD74 ex 6	ROS1 ex 34	CD74{NM_001025159.2}:r.1_812_ROS1{NM_002944.2}:r.5757_7368	COSF1201
EGFR Variant III	EGFR ex 1	EGFR ex 8	EGFR{NM_005228.5}:r.350_1150del	NA
EGFR-SEPT14	EGFR ex 24	SEPT14 ex 10	EGFR{NM_005228.5}:r.1_3207_SEPT14{NM_207366.3}:r.1200_3752	NA
EML4-ALK	EML4 ex 13	ALK ex 20	EML4{NM_019063.4}:r.1_1763_ALK{NM_004304.4}:r.4125_6265	COSF463
ETV6-NTRK3	ETV6 ex 5	NTRK3 ex 15	ETV6{NM_001987.4}:r.1_1283_NTRK3{NM_001012338.2}:r.1892_3004	COSF572
FGFR3-BAIAP2L1	FGFR3 ex 17	BAIAP2L1 ex 2	FGFR3{NM_000142.4}:r.1_2530_BAIAP2L1{NM_018842.4}:r.315_3682	NA
FGFR3-TACC3	FGFR3 ex 17	TACC3 ex 11	FGFR3{NM_000142.4}:r.1_2530_TACC3{NM_006342.3}:r.2066_2799	NA
KIF5B-RET	KIF5B ex 24	RET ex 11	KIF5B{NM_004521.2}:r.1_3231_RET{NM_020975.6}:r.2070_5617	COSF1263
LMNA-NTRK1	LMNA ex 2	NTRK1 ex 10	LMNA{NM_170707.3}:r.1_762_NTRK1{NM_001012331.1}:r.1290_2647	COSF1654
MET ex 14 Skipping	MET ex 13	MET ex 15	MET{NM_001127500.3}:r.3338_3478del	NA
NCOA4-RET	NCOA4 ex 8	RET ex 12	NCOA4{NM_001145260.1}:r.1_1014_RET{NM_020975.6}:r.2327_5617	COSF1492
PAX8-PPARG1	PAX8 ex 9	PPARG1 ex 3	PAX8{NM_003466.4}:r.1_1253_PPARG{NM_138712.3}:r.246_1892	COSF1217
SLC34A-ROS1	SLC34A2 ex 4	ROS1 ex 34	SLC34A2{NM_006424.2}:r.1_460_ROS1{NM_002944.2}:r.5757_7368	NA
SLC45A3-BRAF	SLC45A3 ex 1	BRAF ex 8	SLC45A3{NM_033102.3}:r.1_109_BRAF{NM_004333.5}:r.1206_4560	COSF872
TFG-NTRK1	TFG ex 5	NTRK1 ex 9	TFG{NM_006070.5}:r.1_851_NTRK1{NM_001012331.1}:r.1234_2647	NA
TMPRSS2-ERG	TMPRSS2 ex 1 (5' UTR)	ERG ex 2	TMPRSS2{NM_005656.3}:r.1_78_ERG{NM_004449.4}:r.124_5042	COSF123
TPM3-NTRK1	TPM3 ex 7	NTRK1 ex 9	TPM3{NM_153649.3}:r.1_794_NTRK1{NM_001012331.1}:r.1234_2647	COSF1329