Seraseq®
cfRNA Fusion Mix

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® cfRNA Fusion Mix 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 can provide added confidence in the reliability of results obtained for unknown specimens. The use of independent reference products may provide valuable information concerning assay accuracy and bioinformatics pipeline analysis.

PRINCIPLES OF THE PROCEDURE
Seraseq cfRNA Fusion Mix is ready to use in NGS assays in steps that follow RNA isolation. No further purification or RNA isolation is needed.

REAGENTS

Table 1. Seraseq cfRNA Fusion Mix

Material No.	Product
0710-4078	Seraseq® cfRNA Fusion Mix

Item No. 0710-4078. 1 vial, 25 µL per vial, 10 ng/µL. See Technical Product Report for lot specific information.

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq cfRNA Fusion Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq cfRNA Fusion Mix is manufactured using genomic RNA extracted from the 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). Purified RNA is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer.

Safety Precautions
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. 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 cfRNA Fusion Mix frozen at -70 °C or colder. Once opened, a vial should be aliquoted into single-use aliquots if not consumed on initial thaw.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION
Seraseq cfRNA Fusion Mix is a mixture of human total RNA and synthetic RNA transcripts. 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 cfRNA Fusion Mix consists of total cellular RNA purified from GM24385 cell line and biosynthetic RNA that have been pooled and fragmented to a cfRNA-like fragment size of 180-300 nt. The RNA is in 1 mM Tris HCl, pH 8.0, aqueous buffer. 25 µL is provided per vial and the concentration is 10 ng/µL. See Technical Product Report 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 cfRNA Fusion Mix 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 cfRNA Fusion Mix 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 2. 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 cfRNA Fusion Mix 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 19 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 cfRNA Fusion Mix, however we recommend verifying the product QC release criteria before setting acceptable performance characteristics for this reference standard. 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.

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Table 2 Indicates each of the fusion RNA variants and exon skipping events

LIMITATIONS OF THE PROCEDURE

Seraseq cfRNA Fusion Mix MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

TEST PROCEDURES and INTERPRETATION OF RESULTS 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 does not claim that others can duplicate test results exactly. Seraseq cfRNA Fusion 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 storage conditions or use of outdated product may produce erroneous results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq cfRNA Fusion Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq cfRNA Fusion Mix 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.

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: RNA Fusions Present

RNA Fusion	5' Transcript	5' Partner Exon	5' Breakpoint GRCh37	5' Breakpoint GRCh38	3' Transcript
CCDC6::RET	NM_005436.5	CCDC6 exon 1	10:61665880	10:59906122	NM_020975.6
CD74::ROS1	NM_001025159.3	CD74 exon 6	5:149784243	5:150404680	NM_001378902.1
EGFR Variant III	NM_005228.5	EGFR exon 1	7:55087058	7:55019365	NM_005228.5
EGFR::SEPTIN14	NM_005228.5	EGFR exon 24	7:55268106	7:55200413	NM_207366.3
EML4::ALK	NM_019063.5	EML4 exon 13	2:42522656	2:42295516	NM_004304.5
ETV6::NTRK3	NM_001987.5	ETV6 exon 5	12:12022903	12:11869969	NM_001012338.3
FGFR3::BAIAP2L1	NM_000142.5	FGFR3 exon 17	4:1808661	4:1806934	NM_018842.5
FGFR3::TACC3	NM_000142.5	FGFR3 exon 17	4:1808661	4:1806934	NM_006342.3
KIF5B::RET	NM_004521.3	KIF5B exon 24	10:32306071	10:32017143	NM_020975.6
LMNA::NTRK1	NM_170707.4	LMNA exon 2	1:156100564	1:156130773	NM_002529.4
MET ex 14 Skipping	NM_000245.4	MET exon 13	7:116411708	7:116771654	NM_000245.4
NCOA4::RET	NM_001145263.2	NACC2 exon 7	10:51582939	10:46012883	NM_020975.6
PAX8::PPARG	NM_003466.4	PAX8 exon 9	2:113992971	2:113235394	NM_138711.6
SLC34A2::ROS1	NM_006424.3	SLC34A2 exon 4	4:25665952	4:25664330	NM_001378902.1
SLC45A3::BRAF	NM_033102.3	SLC45A3 exon 1	1:205649522	1:205680394	NM_004333.6
TFG::NTRK1	NM_006070.6	TFG exon 5	3:100451516	3:100732672	NM_002529.4
TMPRSS2::ERG	NM_005656.4	TMPRSS2 exon 1	21:42880008	21:41508081	NM_004449.4
FGFR1::TACC1	NM_023110.2	FGFR1 exon 17	8:38271436	8:38413918	NM_006283.2
FGFR2::CCDC6	NM_000141.4	FGFR2 exon 17	10:123243212	10:121483698	NM_005436.4

NOTE: Above list does not include variants present in the GM24385 background. For a more detailed description of positional information of the fusions, please see the technical spreadsheet posted on the product page.