

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 NTRK Fusion RNA Reference Material is a full-process reference material formulated for use with targeted Next Generation Sequencing (NGS) assays that detect RNA expressed from gene fusions in Neurotrophic Tyrosine Receptor Kinase (NTRK) genes. This product is intended as a quality reference material for translational and disease research testing to monitor nucleic acid extraction, 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.*

REAGENTS

Item No. 0710-1031. One 10 µm FFPE curl per vial.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq FFPE NTRK Fusion RNA 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 FFPE-treated curls are made by treating cells with HistoGel, then fixing them with 10% Formalin, and washing prior to embedding and sectioning.

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 FFPE NTRK Fusion RNA Reference Material beyond the expiration date. Avoid contamination of the product when opening and closing the vial.

STORAGE INSTRUCTIONS

Store Seraseq FFPE NTRK Fusion RNA Reference Material at 2-8°C. Shelf life when stored under these conditions is two years from date of manufacture.

PROCEDURE

Materials Provided

Seraseq FFPE NTRK Fusion RNA Reference Material consists of engineered cells which have been formalin treated and embedded in paraffin to create an FFPE block, which is then sliced into 10 µm sections. One 10 µm FFPE curl is provided per vial.

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Seraseq FFPE NTRK Fusion RNA Reference Material must go through an extraction process. Refer to your usual assay procedures in order to determine the amount of extracted material to use in library preparation.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

RNA Yield and Quality

Seraseq FFPE NTRK Fusion RNA Reference Material is compatible with different commercially available nucleic acid extraction methods commonly used for FFPE specimens. Each FFPE curl yields >300 ng of extractable RNA when using Agencourt® Fomasure extraction kit and quantifying RNA by Thermo Fisher Qubit RNA HS assay.

Fusion RNAs Present in the Product

Table 1 indicates each of the fusion RNAs represented in Seraseq FFPE NTRK Fusion RNA Reference Material. The fusion RNA species in this product are NOT present at the DNA level. Detection of fusion RNAs may differ across different NGS panels and different test reagent lots. While the presence of each fusion RNA in this product is confirmed during manufacturing using functional NGS and/or digital PCR-based fusion RNA assays, there may be apparent differences in observed fusion levels due to assay characteristics. Seraseq FFPE NTRK Fusion RNA Reference Material does not have assigned values for the ratios of fusion transcripts to wild-type transcripts for the same genes, or for the overall quantity of fusion transcripts. Each laboratory must establish an assay-specific expected value for each fusion and each lot of Seraseq FFPE NTRK Fusion RNA Reference Material. 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 online at www.seracare.com/oncology.

LIMITATIONS OF THE PROCEDURE

Seraseq FFPE NTRK Fusion RNA Reference Material 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 FFPE NTRK Fusion RNA Reference Material is not a calibrator and should not be used for assay calibration. 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. Fusion RNAs present in Seraseq FFPE NTRK Fusion RNA Reference Material

| Fusion RNA | 5' Partner | 3' Partner | HGVS Name |
|---------------|----------------|---------------|----------------------------------------------------------------------|
| TPM3-NTRK1 | TPM3 exon 7 | NTRK1 exon 10 | TPM3{ENST00000368533}:r.1_717_NTRK1{ENST00000392302}:r.1262_2609 |
| LMNA-NTRK1 | LMNA exon 11 | NTRK1 exon 11 | LMNA{ENST00000368299}:r.1:2015_NTRK1{ENST00000392302}:r.1318_2609 |
| IRF2BP2-NTRK1 | IRF2BP2 exon 1 | NTRK1 exon 10 | IRF2BP2{ENST00000366609}:r.1_1079_NTRK1{ENST00000392302}:r.1262_2609 |
| SQSTM1-NTRK1 | SQSTM1 exon 5 | NTRK1 exon 10 | SQSTM1{ENST00000389805}:r.1_932_NTRK1{ENST00000392302}:r.1262_2609 |
| TFG-NTRK1 | TFG exon 5 | NTRK1 exon 10 | TFG{ENST00000240851}:r.1_920_NTRK1{ENST00000392302}:r.1262_2609 |
| AFAP1-NTRK2 | AFAP1 exon 14 | NTRK2 exon 12 | AFAP1{ENST00000420658.1}:r.1_2170_NTRK2{ENST00000376214}:r.2098_5608 |
| NACC2-NTRK2 | NACC2 exon 4 | NTRK2 exon 13 | NACC2{ENST00000371753}:r.1_1314_NTRK2{ENST00000376214}:r.2134_5608 |
| QKI-NTRK2 | QKI exon 6 | NTRK2 exon 16 | QKI{ENST00000361752}:r.1_1485_NTRK2{ENST00000376214}:r.2383_5608 |
| TRIM24-NTRK2 | TRIM24 exon 12 | NTRK2 exon 15 | TRIM24{ENST00000343526}:r.1_2229_NTRK2{ENST00000376214}:r.2335_5608 |
| PAN3-NTRK2 | PAN3 exon 1 | NTRK2 exon 17 | PAN3{ENST00000380958}:r.1_582_NTRK2{ENST00000376214}:r.2572_5608 |
| ETV6-NTRK3 | ETV6 exon 5 | NTRK3 exon 14 | ETV6{ENST00000396373}:r.1_1283_NTRK3{ENST00000394480}:r.1719_19984 |
| ETV6-NTRK3 | ETV6 exon 5 | NTRK3 exon 15 | ETV6{ENST00000396373}:r.1_1283_NTRK3{ENST00000394480}:r.1908_19984 |
| ETV6-NTRK3 | ETV6 exon 4 | NTRK3 exon 15 | ETV6{ENST00000396373}:r.1_737_NTRK3{ENST00000394480}:r.1908_19984 |
| ETV6-NTRK3 | ETV6 exon 4 | NTRK3 exon 14 | ETV6{ENST00000396373}:r.1_737_NTRK3{ENST00000394480}:r.1719_19984 |
| BTBD1-NTRK3 | BTBD1 exon 4 | NTRK3 exon 14 | BTBD1{ENST00000261721}:r.1_1065_NTRK3{ENST00000394480}:r.1719_19984 |

Exon numbers correspond to ENST00000392302.6 for NTRK1

Exon numbers correspond to ENST00000376214.5 for NTRK2

Exon numbers correspond to ENST00000394480.6 for NTRK3