

(US) Package Insert

FOR IN VITRO DIAGNOSTIC USE.

Intended Use

The TruSight™ Oncology RNA Control is intended for qualitative *in vitro* diagnostic use as a quality control to monitor analytical performance of the library preparation, sequencing, and analysis steps of Next Generation Sequencing (NGS) based molecular diagnostic assays used for the detection of select RNA variants. This product is also intended to help monitor performance of an NGS test system by detecting analytical deviations such as those that may arise from reagent or instrument variation in genetic testing.

Product Description

The TruSight Oncology (TSO) RNA Control is a multiplexed blend of RNA transcripts in a background of total RNA from the GM24385 cell line. It contains 16 fusions across 26 genes and two splice variants across two genes ([Table 1](#)).

Table 1 Variants Present in TSO RNA Control

Variant		
CCDC6-RET	FGFR3-BAIAP2L1	PAX8-PPARG
CD74-ROS1	FGFR3-TACC3	SLC34A2-ROS1
EGFR-SEPT14	KIF5B-RET	SLC45A3-BRAF
EGFR VIII*	LMNA-NTRK1	TFG-NTRK1
EML4-ALK	MET Exon 14*	TMPRSS2-ERG
ETV6-NTRK3	NCOA4-RET	TPM3-NTRK1

* EGFR VIII and MET Exon 14 are splice variants. All other variants are gene fusions.

Limitations

For *in vitro* diagnostic use.

- Results presented in the labeling were obtained with representative assays. Performance characteristics are provided for information purposes only. Variant detection results of the TruSight Oncology RNA Control might differ according to the library preparation method, sequencing method, and the bioinformatics pipeline. The end user is responsible for establishing their own performance criteria appropriate for their system.

Product Components

Product	Catalog Number	Quantity	Volume	Concentration*	Active Ingredients	Storage Temperature
TruSight Oncology RNA Control	20065042	1	25 µl	25 ng/µL	Synthetic RNA pool	-85°C to -65°C

* Minimum concentration is indicated. Actual concentration varies per lot and is indicated on the tube label.

Storage and Handling

- TSO RNA Control, when stored at -85°C to -65°C, is stable through the expiration date printed on the tube label and on the kit box. The tube can undergo 10 freeze-thaws from multiple uses of the tube. Use good laboratory practices to avoid contamination.
- Do not aliquot.

Warnings and Precautions



CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.

- Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For environmental, health, and safety information, refer to the safety data sheets (SDS) at support.illumina.com/sds.html.
- Changes in the physical appearance of the reagents can indicate deterioration of the materials. If changes in the physical appearance occur (for example, changes in reagent color or cloudiness), do not use the reagents.
- Avoid cross-contamination.
 - Follow proper laboratory practices when handling the product.
 - Use fresh consumable labware and fresh pipette tips between samples and between dispensing controls.
 - Use aerosol resistant tips to reduce the risk of cross-contamination.
- Follow proper assay procedure and note safety, laboratory, and assay warnings and precautions.

- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling the product. Wash hands thoroughly after handling the product.
- Use nuclease-free microcentrifuge tubes, plates, pipette tips, and reservoirs.
- Use precision pipettes to ensure accurate product delivery. Calibrate regularly according to manufacturer specifications.
- Do not use the TSO RNA Control beyond the expiration date on the tube label.

Instructions for Use

NOTE Use QC materials (TSO RNA Control) in accordance with local, state, and/or federal regulations or accreditation requirements.

1. Thaw the contents on ice.
2. Gently vortex or invert the tube to mix, then briefly centrifuge the tube to collect the contents to the bottom of the tube.
3. Dilute to the desired concentration in an appropriate diluent. Use the actual concentration on the tube label for a given lot of control when making dilution calculations, if dilutions are needed.
 - A suggested diluent for the TSO RNA Control is DNase- and RNase- free water.
4. Test the control like a patient sample in the assay workflow.
5. Store at label conditions between uses.

Performance Characteristics

The TSO RNA Control was tested using TruSight Oncology Comprehensive (TSO Comprehensive, hybrid-capture enrichment-based), NYU FUSION-SEQer assay (amplicon-based), and the OncoPrint Focus assay (amplicon-based).

Reproducibility

The TSO RNA Control was tested in a reproducibility study using TSO Comprehensive as a representative assay. The TSO RNA Control was diluted in RNase- and DNase-free water and 40 ng was used as sample input. At each of three external sites, two operators per site tested three lots of the TSO RNA Control with four lots of TSO Comprehensive assay kits. Libraries were sequenced on NextSeq 550Dx instruments. In total, 96 sample results were generated for the TSO RNA Control. There were 13 calls per sample for a total of 1248 evaluable expected calls.

A representative set of variants were selected for evaluation of variant detection rate in the TSO RNA Control (Table 2). This set of variants span a range of cancer related genes and encompass multiple fusions and a splice variant.

Table 2 Selected TSO RNA Control Variants for Detection with TSO Comprehensive Assay

Variant			
CCDC6-RET	FGFR3-BAIAP2L1	SLC45A3-BRAF	MET Exon 14*
CD74-ROS1	KIF5B-RET	TFG-NTRK1	
EML4-ALK	NCOA4-RET	TMPRSS2-ERG	
ETV6-NTRK3	PAX8-PPARG	TPM3-NTRK1	

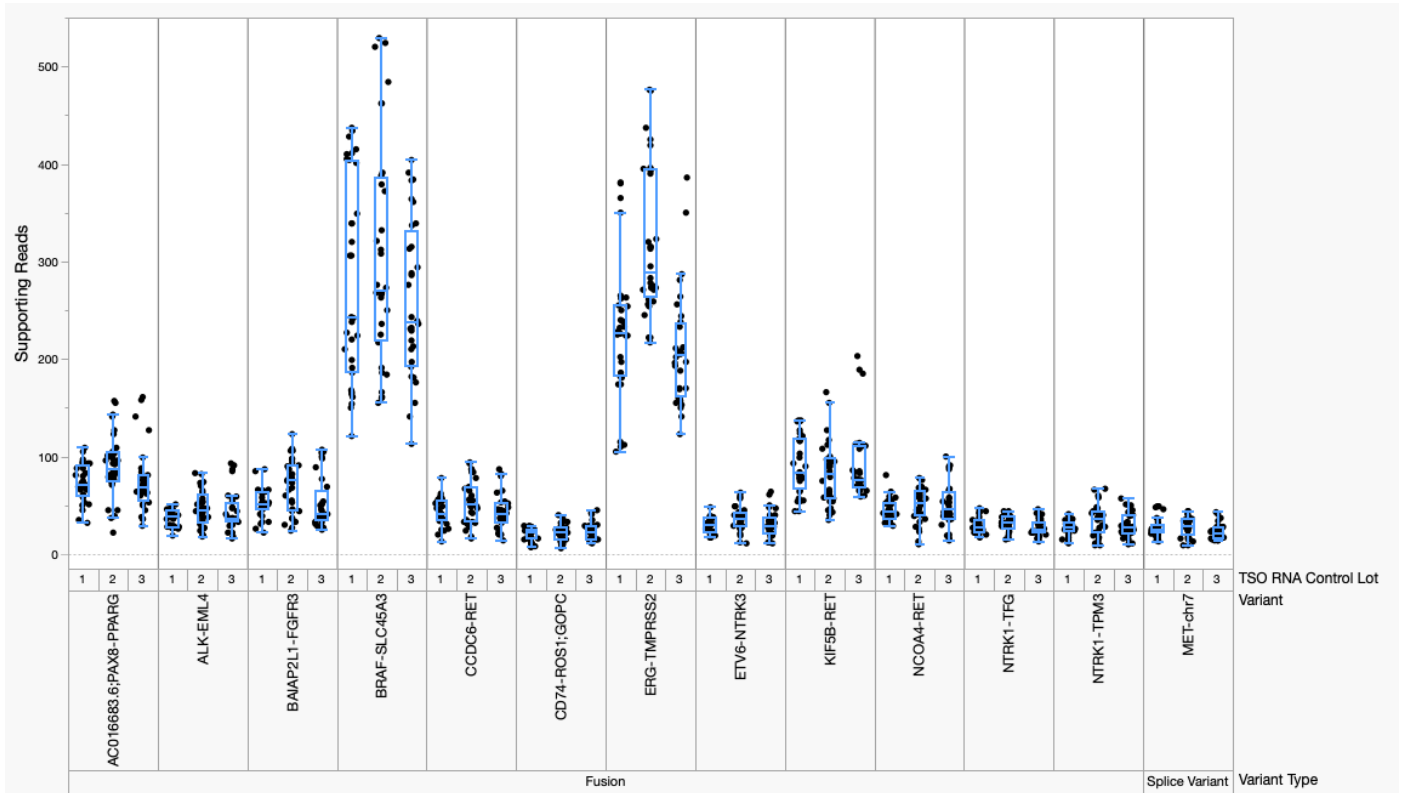
* MET Exon 14 is a splice variant. All other variants are gene fusions.

Table 3 summarizes the percentage of observed positive calls. Correct calls were based on detection of the 13 variants in Table 2. Figure 1 demonstrates the lot-to-lot consistency of supporting reads for each fusion and splice variant.

Table 3 External Site Evaluation of TSO RNA Control with TSO Comprehensive Assay

Site	Operator	Number of Runs	Total Expected Calls	Observed Positive Calls	% Positive Calls
S1	1	8	208	208	100%
S1	2	8	208	208	100%
S2	3	8	208	208	100%
S2	4	8	208	207	100%
S3	5	8	208	206	99%
S3	6	8	208	208	100%
Total	All	48	1248	1245	99.8%

Figure 1 TSO RNA Control Fusions and Splice Variant Lot-to-Lot Consistency Across Three Sites



Evaluation by NYU FUSION-SEQer and Oncomine Focus

The TSO RNA Control was tested with NYU FUSION-SEQer assay and Oncomine Focus assay at a single site. The TSO RNA Control was used as sample input (125 ng for NYU FUSION-SEQer assay, 20 ng for Oncomine Focus assay). Three lots of the TSO RNA Control were tested in duplicate or in triplicate in four sequencing runs. Libraries were sequenced on the Illumina NextSeq or the Ion GeneStudio System. In total, 27 sample results were generated for the TSO RNA Control for each assay.

The expected results as a detection rate (%) of fusions and splice variants in the TSO RNA Control are summarized [Table 4](#). [Table 4](#) also includes the data from the reproducibility study using TSO Comprehensive. Detection of the MET splice variant varied depending on the amplicon assay being run.

Table 4 Detection Rate (%) of TSO RNA Control Fusions and Splice Variants (NE: Not Evaluated by the Assay)

Fusion Gene A	Fusion Gene B	TSO Comprehensive			TSO Comprehensive			TSO Comprehensive			NYU FUSION-SEQer			Oncomine Focus		
		Site 1			Site 2			Site 3			Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3						
Total Number of Samples Tested		16	8	8	8	16	8	8	8	16	9	9	9	9	9	9
AC016683.6;PAX8	PPARG	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ALK	EML4	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
BAIAP2L1	FGFR3	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
BRAF	SLC45A3	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CCDC6	RET	100	100	100	100	100	100	100	100	100	100	100	100	89	100	89
CD74	ROS1;GOPC	100	100	100	88	100	100	100	100	100	100	100	100	100	100	89
EGFR	SEPT14	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
ERG	TMPRSS2	100	100	100	100	100	100	100	100	100	100	100	100	89	89	89
ETV6	NTRK3	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
FGFR3	TACC3	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
LMNA	NTRK1	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
KIF5B	RET	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NCOA4	RET	100	100	100	100	100	100	100	100	100	100	100	100	100	100	89
NTRK1	TFG	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NTRK1	TPM3	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ROS1;GOPC	SLC34A2	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100

Splice Variant	Chromosome	TSO Comprehensive			TSO Comprehensive			TSO Comprehensive			NYU FUSION-SEQer			Oncomine Focus		
		Site 1			Site 2			Site 3			Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3						
Total Number of Samples Tested		16	8	8	8	16	8	8	8	16	9	9	9	9	9	9
EGFR	chr7	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
MET	chr7	100	100	100	100	100	100	100	75	100	100	100	100	89	33	44

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Revision History

Document	Date	Description of Change
Document # 200060398 v00	September 2024	Initial release.

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Contact Information



Illumina, Inc.
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com



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