TruSight Oncology DNA Control

illumina®

(US) Package Insert

FOR IN VITRO DIAGNOSTIC USE.

Intended Use

The TruSight™ Oncology DNA 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 DNA 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) DNA Control is a multiplexed blend of biosynthetic DNA in a background of genomic DNA from the GM24385 cell line. It contains 40 variants across 28 genes representing single nucleotide variants (SNV), insertions, deletions, and rearrangements (Table 1).

Table 1 Variants Present in TSO DNA Control

COSMIC ID	Gene	Nucleotide Change	Amino Acid Change	Variant Type
COSM33765	AKT1	c.49G>A	p.E17K	SNV
COSM13127	APC	c.4348C>T	p.R1450*	SNV
COSM18561	APC	c.4666dup	p.T1556Nfs*3	INSERTION
COSM21924	ATM	c.1058_1059del	p.C353Sfs*5	DELETION
COSM476	BRAF	c.1799T>A	p.V600E	SNV
COSM5664	CTNNB1	c.121A>G	p.T41A	SNV
COSM12378	EGFR	c.2310_2311insGGT	p.D770_N771insG	INSERTION
COSM6225	EGFR	c.2236_2250del	p.E746_A750del	DELETION
COSM6224	EGFR	c.2573T>G	p.L858R	SNV
COSM6240	EGFR	c.2369C>T	p.T790M	SNV
COSM20959	ERBB2	c.2313_2324dup	p.Y772_A775dup	INSERTION
COSM715	FGFR3	c.746C>G	p.S249C	SNV
COSM783	FLT3	c.2503G>T	p.D835Y	SNV
COSM33661	FOXL2	c.402C>G	p.C134W	SNV

COSMIC ID	Gene	Nucleotide Change	Amino Acid Change	Variant Type
COSM52969	GNA11	c.626A>T	p.Q209L	SNV
COSM28758	GNAQ	c.626A>C	p.Q209P	SNV
COSM27887	GNAS	c.2530C>T	p.R844C	SNV
COSM28747	IDH1	c.394C>T	p.R132C	SNV
COSM12600	JAK2	c.1849G>T	p.V617F	SNV
COSM1314	KIT	c.2447A>T	p.D816V	SNV
COSM521	KRAS	c.35G>A	p.G12D	SNV
COSM18918	MPL	c.1544G>T	p.W515L	SNV
COSM17559	NPM1	c.860_863dup	p.W288Cfs*12	INSERTION
COSM584	NRAS	c.182A>G	p.Q61R	SNV
COSM736	PDGFRA	c.2525A>T	p.D842V	SNV
COSM28053	PDGFRA	c.1694_1695insA	p.S566Qfs*6	INSERTION
COSM763	PIK3CA	c.1633G>A	p.E545K	SNV
COSM775	PIK3CA	c.3140A>G	p.H1047R	SNV
COSM12464	PIK3CA	c.3204_3205insA	p.*1069Mext*3	INSERTION
COSM5809	PTEN	c.800del	p.K267Rfs*9	DELETION
COSM4986	PTEN	c.741dup	p.P248Tfs*5	INSERTION
COSM965	RET	c.2753T>C	p.M918T	SNV
COSM14105	SMAD4	c.1394dup	p.A466Gfs*28	INSERTION
COSM6530	TP53	c.723del	p.C242Afs*5	DELETION
COSM10648	TP53	c.524G>A	p.R175H	SNV
COSM10662	TP53	c.743G>A	p.R248Q	SNV
COSM10660	TP53	c.818G>A	p.R273H	SNV
COSM18610	TP53	c.267del	p.S90Pfs*33	DELETION
Not applicable	NCOA4-RET	Not applicable	Not applicable	Gene Rearrangement (Translocation)
Not applicable	TPR-ALK	Not applicable	Not applicable	Gene Rearrangement (Translocation)

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 DNA 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.
- Illumina® has not evaluated detection of NCOA4-RET and TPR-ALK in the TruSight Oncology DNA Control.

Product Components

Product	Catalog Number	Quantity	Volume	Concentration*	Active Ingredients	Storage Temperature
TruSight Oncology DNA Control	20065041	1	25 µl	20 ng/µL	Synthetic DNA pool	-25°C to -15°C

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

Storage and Handling

- TSO DNA Control, when stored at -25°C to -15°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 DNA Control beyond the expiration date on the tube label.

Instructions for Use

NOTE Use QC materials (TSO DNA 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 DNA Control is Tris-EDTA (10 mM Tris, 1 mM EDTA, pH 8.0).
4. Test the control like a patient sample in the assay workflow.
5. Store at label conditions between uses.

Performance Characteristics

The TSO DNA Control was tested using TruSight Oncology Comprehensive (TSO Comprehensive, hybrid-capture enrichment-based), NYU Langone Genome PACT assay (hybrid-capture enrichment-based), and the Oncomine Focus assay (amplicon-based).

Reproducibility

The TSO DNA Control was tested in a reproducibility study using TSO Comprehensive as a representative assay. The TSO DNA Control was diluted in Tris-EDTA buffer and 40 ng was used as sample input. At each of three external sites, two operators per site tested three lots of the TSO DNA Control with three lots of TSO Comprehensive assay kits. Libraries were sequenced on NextSeq 550Dx instruments. In total, 112 sample results were generated for the TSO DNA Control. There were 24 calls per sample for a total of 2688 evaluable expected calls.

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

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

Variant			
AKT1 E17K	EGFR E746_A750del	KIT D816V	PTEN P248Tfs*5
APC R1450*	ERBB2 Y772_A775dup	KRAS G12D	RET M918T
APC T1556Nfs*3	GNA11 Q209L	MPL W515L	SMAD4 A466Gfs*28
ATM C353Sfs*5	GNAQ Q209P	NRAS Q61R	TP53 R175H
CTNNB1 T41A	GNAS R844C	PDGFRA D842V	TP53 R248Q
EGFR L858R	JAK2 V617F	PIK3CA E545K	TP53 R273H

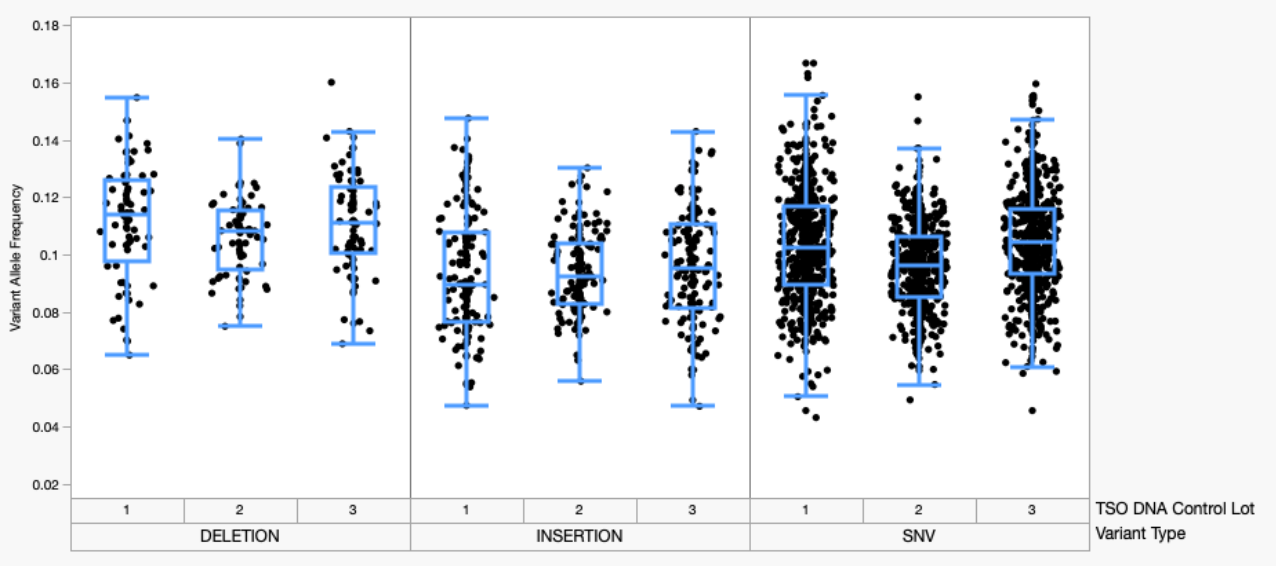
Table 3 summarizes the percentage of observed positive calls. Correct calls were based on detection of the 24 variants in Table 2. Figure 1 demonstrates the lot-to-lot consistency of variant allele frequency by variant class.

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

Site	Site Operator	Number of Runs	Total Expected Calls	Observed Positive Calls	% Positive Calls
1	1	3	432	410	94.9%
1	2	3	432	408	94.4%
2	1	3	432	432	100%
2	2	3	432	432	100%
3	1	4	528*	528	100%
3	2	3	432	432	100%
Total	All	19	2688	2642	98.3%

* Four additional replicates due to repeat run.

Figure 1 TSO DNA Control Small Variants Lot-to-Lot Consistency Across Three Sites



Evaluation by NYU Langone Genome PACT and Oncomine Focus

The TSO DNA Control was tested with the NYU Langone Genome PACT (NYU PACT) assay and the Oncomine Focus assay at a single site. The TSO DNA Control was used as sample input (100 ng for NYU PACT assay, 20 ng for Oncomine Focus assay). Three lots of the TSO DNA 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 DNA Control for each assay.

The expected results as a detection rate (%) of small variants in the TSO DNA Control are summarized in Table 4. Table 4 also includes data from the reproducibility study using TSO Comprehensive.

Table 4 Detection Rate (%) of TSO DNA Control Small Variants (NE: Not Evaluated by the Assay)

Variant Class	Gene	Nucleotide Change	Amino Acid Change	TSO Comprehensive Site 1			TSO Comprehensive Site 2			TSO Comprehensive Site 3			NYU Langone Genome PACT			Oncomine Focus		
				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			12	12	12	12	12	12	14	12	14	9	9	9	9	9	9
Deletion	ATM	c.1058_1059del	p.C353Sfs*5	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	EGFR	c.2236_2250del	p.E746_A750del	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	PTEN	c.800del	p.K267Rfs*9	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
	TP53	c.267del	p.S90Pfs*33	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
	TP53	c.723del	p.C242Afs*5	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
Insertion	APC	c.4666dup	p.T1556Nfs*3	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	EGFR	c.2310_2311insGGT	p.D770_N771insG	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
	ERBB2	c.2313_2324dup	p.Y772_A775dup	92	92	100	100	100	100	100	100	100	100	89	100	100	100	100
	NPM1	c.860_863dup	p.W288Cfs*12	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	89	100	NE	NE	NE
	PDGFRA	c.1694_1695insA	p.S566Qfs*6	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
	PIK3CA	c.3204_3205insA	p.*1069Mext*3	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
	PTEN	c.741dup	p.P248Tfs*5	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	SMAD4	c.1394dup	p.A466Gfs*28	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE

Variant Class	Gene	Nucleotide Change	Amino Acid Change	TSO Comprehensive Site 1			TSO Comprehensive Site 2			TSO Comprehensive Site 3			NYU Langone Genome PACT			Oncomine Focus		
				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
SNV	AKT1	c.49G>A	p.E17K	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	APC	c.4348C>T	p.R1450*	92	92	100	100	100	100	100	100	100	100	78	100	NE	NE	NE
	BRAF	c.1799T>A	p.V600E	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	78	100	78	89	100
	CTNNB1	c.121A>G	p.T41A	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	EGFR	c.2573T>G	p.L858R	92	92	100	100	100	100	100	100	100	100	89	100	100	100	100
	EGFR	c.2369C>T	p.T790M	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	89	100	100	100	100
	FGFR3	c.746C>G	p.S249C	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
	FLT3	c.2503G>T	p.D835Y	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
	FOXL2	c.402C>G	p.C134W	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	NE	NE	NE
	GNA11	c.626A>T	p.Q209L	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	GNAQ	c.626A>C	p.Q209P	92	92	100	100	100	100	100	100	100	100	89	100	100	100	100
	GNAS	c.2530C>T	p.R844C	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	IDH1	c.394C>T	p.R132C	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	78	100	100	100	100
	JAK2	c.1849G>T	p.V617F	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	KIT	c.2447A>T	p.D816V	92	92	100	100	100	100	100	100	100	100	89	100	100	100	100
	KRAS	c.35G>A	p.G12D	92	92	100	100	100	100	100	100	100	100	78	100	100	100	100
	MPL	c.1544G>T	p.W515L	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	NRAS	c.182A>G	p.Q61R	92	92	100	100	100	100	100	100	100	100	78	100	100	100	100
	PDGFRA	c.2525A>T	p.D842V	92	92	100	100	100	100	100	100	100	100	89	100	100	100	100
	PIK3CA	c.1633G>A	p.E545K	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	PIK3CA	c.3140A>G	p.H1047R	NE	NE	NE	NE	NE	NE	NE	NE	NE	100	100	100	100	100	100
	RET	c.2753T>C	p.M918T	92	92	100	100	100	100	100	100	100	100	100	100	100	100	100
	TP53	c.524G>A	p.R175H	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	TP53	c.743G>A	p.R248Q	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE
	TP53	c.818G>A	p.R273H	92	92	100	100	100	100	100	100	100	100	100	100	NE	NE	NE

Revision History

Document	Date	Description of Change
Document # 200052548 v01	September 2024	Launch release of TSO DNA Control PI.
Document # 200052548 v00	June 2024	Initial release.

Patents and Trademarks

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Product Labeling

For a complete reference of symbols that appear on product packaging and labeling, refer to the symbol key at support.illumina.com on the *Documentation* tab for your kit.