PanCancer cfDNA Reference Standard (SID-000178) – Instructions for use

For Research Use Only

SensID - High-Quality Reference Materials

R&D needs references and controls. SensID GmbH manufactures High Quality Reference Materials / Controls for Molecular Diagnostic R&D.
Our mission is to provide certified standards ready for your needs in the highest quality to ease your processes.
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Content

Product	Catalog No.
PanCancer cfDNA Reference Standard	SID-000178
Target concentration 10 ng/µl and target quantity 250 ng dsDNA. For exact amount and concentration of each batch measured by QC, refer to the Certificate of Analysis (CoA) of the respective lot.	

Symbols

REF	Catalog number
LOT	Lot number
	Use by
	Legal manufacturer
	Not for reuse
	Temperature limitations

Storage

The product should be stored at 2 °C to 8 °C upon arrival. DO NOT FREEZE. The product is solved in TE buffer (Tris-EDTA (10 mM Tris, 1 mM EDTA), pH 8,0, and stable until the expiration date when stored under these conditions.

Intended Use

Platform-agnostic one-vial pan-cancer multiplex ctDNA reference standard containing 80 tumor-relevant variants across 33 genes (50 SNV, 11 Indels, 19 fusions) in highly characterized human DNA from a tumor cell line. It was designed for use in:

1. Spike-in experiments
2. Verification of mutation detection
3. Process verification and validation
4. Validation and development of targeted sequencing protocols (whole genome sequencing, amplicon sequencing) and PCR protocols
5. Challenging bioinformatics pipelines

This product is for research use only and is not intended for clinical or diagnostic use.

Protocol: PanCancer cfDNA Reference Standard

Important information to note prior to product use:

- Centrifuge SID-000178 briefly to avoid liquid holding back in the lid of the vial!
- Work in a clean environment (e.g. laminar flow hood) to avoid contamination of the product
- Do not vortex! Mix by pipetting up and down 10 times to obtain a homogeneous suspension.
- DNA purified from a tumor cell line. No further purification or DNA isolation steps needed
- The purified DNA is present in cfDNA (human) at a fragment size of 167 bp ±15 %
- While the presence and frequency of each mutation and/or amplification in this product is evaluated during manufacture using dPCR[1] assay, there may be differences in observed allele frequencies due to specific assay characteristics.

Technical Assistance

Our Technical Service Assistance is staffed by experienced scientists with extensive practical and theoretical expertise with our products. If you have any questions or experience any difficulties regarding the PanCancer cfDNA Reference Standard or SensID GmbH products in general, please do not hesitate to contact us.
SensID GmbH customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at SensID GmbH. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.
For technical assistance and more information, please see our Website www.sens-id.com or call one of the SensID GmbH Technical Service Assistance.

Product Use limitations

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components. Check primary packaging before first opening. Do not use products from damaged primary packaging.

Quality Control

In accordance with SensID's Quality Management System, each lot of PanCancer cfDNA Reference Standard is tested against predetermined specifications to ensure consistent product quality.
The product should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

Warnings and precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
Avoid contamination of the product when opening and closing the vial.
CAUTION: Handle as though it is capable of transmitting infectious agents. This product is formulated using an epithelial, lymphoblast female tumor cell line.

Equipment and Reagents to Be Supplied by User

- Pipets (adjustable)[2]
- Sterile pipet tips with filters

Information on cellular background

Please note that in addition to the variants specified from Table 1 onward, additional genomic variants and copy number alterations are present in the tumor-cell-line background that may generate a high apparent tumor mutational burden (TMB) signal.

[1] Whale, Alexandra S., et al. "Assessment of digital PCR as a primary reference measurement procedure to support advances in precision medicine." Clinical chemistry 64.9 (2018): 1296-1307.

Whale et al. (2018) validated dPCR as an SI-traceable reference measurement procedure based on enumeration and demonstrates how it can be applied for assignment of copy number concentration and fractional abundance values to DNA reference materials in an aqueous solution. Use of dPCR is accepted to define a ground truth for traceable standardization of molecular diagnostic procedures needed for advancements in precision medicine.

[2] Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Table 1 Variants present in the SensID PanCancer cfDNA Reference Standard. HGVS = Human Genome Variation Society

Gene	COSMIC ID	Type of mutation	Amino acid change	HGVS Nomenclature	Localisation in Genome (GRCh38)
AKT1	COSV62571334	Substitution	p.E17K	c.49G>A	chr14: g.104780214C>T
AR	COSV65952830	Substitution	p.H875Y	c.2623C>T	chrX: g.67723701C>T
AR	COSV65953052	Substitution	p.L702H	c.2105T>A	chrX: g.67711621T>A
AR	COSV65956522	Substitution	p.W742L	c.2225G>T	chrX: g.67717529G>T
ATM	COSV53723836	Substitution	p.R337C	c.1009C>T	chr11: g.108247071C>T
ATM	COSV53730980	Substitution	p.R3008C	c.9022C>T	chr11: g.108365359C>T
BRAF	COSV56061424	Substitution	p.G469A (II)	c.1406G>C	chr7: g.140781602C>G
BRAF	COSV56056643	Substitution	p.V600E	c.1799T>A	chr7: g.140753336A>T
BRCA1	COSV105897801	Substitution	p.C61G	c.181T>G	chr17: g.43106487A>C
BRCA1	COSV58784738	Deletion	p.K654Sfs*47	c.1961del	chr17: g.43093577del
BRCA2	COSV105932304	Deletion	p.Ser1722fs / p.S1722Yfs*4	c.5164_5165del	chr13: g.32339519_32339520del
BRCA2	COSV66450275	Deletion	p.R2645Nfs*3	c.7934delG	chr13: g.32362651del
CDK12	COSV70999956	Substitution	p.W719*	c.2157G>A	chr17: g.39492799C>T
CDK12	COSV71002236	Insertion	p.E928_L929insYTKI	c.2784_2785insTACAC AAAGATA	chr17: g.39515746_39515747ins
CHEK2	COSV105248037	Substitution	p.E321K	c.961G>A	chr22: g.28699885C>T
CHEK2	COSV60415074	Substitution	p.K373E	c.1117A>G	chr22: g.28695852T>C
CHEK2	COSV104407895	Substitution	p.R117G	c.349A>G	chr22: g.28725338T>C
EGFR	COSV51765119	Deletion	p.E746_A750del	c.2235_2249del	chr7: g.55174772-55174786del
EGFR	COSV51765161	Substitution	p.L858R	c.2573T>G	chr7: g.55191822T>G
EGFR	COSV51765492	Substitution	p.T790M	c.2369C>T	chr7: g.55181378C>T
ERBB2 (HER2)	COSV54062926	Substitution	p.R678Q	c.2033G>A	chr17: g.39723405G>A
ERBB2 (HER2)	COSV54062780	Substitution	p.L755S	c.2264T>C	chr17: g.39723967T>C
ERBB2 (HER2)	COSV54062198	Substitution	p.S310F	c.929C>T	chr17: g.39711955C>T
ERBB2 (HER2)	COSV54062409	Duplication	p.E770_A771insAYVM / p.Y772_A775dup	c.2313_2324dup	chr17: g.39724728-39724729ins
ESR1	COSV52782264	Substitution	p.E380Q	c.1138G>C	chr6: g.152011697G>C
ESR1	COSV52784970	Substitution	p.S463P	c.1387T>C	chr6: g.152094402T>C
ESR1	COSV52789441	Deletion	p.V422del	c.1265_1267del	chr6: g.152061020_152061022del
ESR1	COSV52781024	Substitution	p.D538G	c.1613A>G	chr6: g.152098791A>G
FGFR3	COSV53390174	Substitution	p.Y373C	c.1118A>G	chr4: g.1804372A>G

Gene	COSMIC ID	Type of mutation	Amino acid change	HGVS Nomenclature	Localisation in Genome (GRCh38)
FGFR3	COSV53390026	Substitution	p.S249C	c.746C>G	chr4: g.1801841C>G
IDH1	COSV61615239	Substitution	p.R132H	c.395G>A	chr2: g.208248388C>T
IDH2	COSV57468751	Substitution	p.R140Q	c.419G>A	chr15: g.90088702C>T
KIT	COSV55386625	Duplication	p.A502_Y503dup	c.1504_1509dup	chr4: g.54726019-54726020
KIT	COSV55386424	Substitution	p.D816V	c.2447A>T	chr4: g.54733155A>T
KRAS	COSV55498802	Substitution	p.Q61H	c.183A>C	chr12: g.25227341T>G
KRAS	COSV55497469	Substitution	p.G12C	c.34G>T	chr12: g.25245351C>A
MET	COSV59256681	Substitution	-	c.3082+1G>T	chr7: g.116771990G>T
NRAS	COSV54736340	Substitution	p.Q61R	c.182A>G	chr1: g.114713908T>C
NTRK1	COSV100693352	Substitution	p.G595R	c.1783G>A	chr1: g.156876550G>A
PALB2	COSV104552889	Substitution	p.W1038*	c.3113G>A	chr16: g.23621362C>T
PALB2	COSV55161955	Deletion	p.M296*	c.886del	chr16: g.23635666Tdel
PDGFRA	COSV57264120	Substitution	p.D842V	c.2525A>T	chr4: g.54285926A>T
PDGFRA	COSV57264866	Substitution	p.N659K	c.1977C>G	chr4: g.54277981C>G
PIK3CA	COSV54062926	Substitution	p.C420R	c.1258T>C	chr3: g.179210192T>C
PIK3CA	COSV55873239	Substitution	p.E545K	c.1633G>A	chr3: g.179218303G>A
PIK3CA	COSV55873195	Substitution	p.H1047R	c.3140A>G	chr3: g.179234297A>G
POLD1	COSV70957772	Substitution	p.D402N	c.1204G>A	chr19: g.50403559G>A
POLD1	COSV101486838	Substitution	p.E318K	c.952G>A	chr19: g.50402723G>A
POLD1	COSV70955559	Substitution	p.S478N	c.1433G>A	chr19: g.50406456G>A
POLE	COSV57676684	Substitution	p.F367S	c.1100T>C	chr12: g.132675741A>G
POLE	COSV57677068	Substitution	p.V411L	c.1231G>T	chr12: g.132673703C>A
POLE	COSV57673247	Substitution	p.P286R	c.857C>G	chr12: g.132676598G>C
PTEN	COSV64288376	Substitution	p.R130Q	c.389G>A	chr10: g.87933148G>A
PTEN	COSV64297648	Deletion	p.E73Dfs*25	c.219_222del	chr10: g.87931055_87931058del
PTEN	COSV64288418	Deletion	p.T319*	c.955_958del	chr10: g.87961047-87961050del
RAD51C	COSV61675984	Substitution	p.R258H	c.773G>A	chr17: g.58709926G>A
STK11	COSV58819912	Substitution	p.F354L	c.1062C>G	chr19: g.1223126C>G
STK11	COSV58819862	Substitution	p.Q37*	c.109C>T	chr19: g.1207022C>T
STK11	COSV58820630	Substitution	p.P281Rfs*6	c.842del	chr19: g.1221320del
TP53	COSV52661038	Substitution	p.R175H	c.524G>A	chr17: g.7675088C>T
TP53	COSV52660980	Substitution	p.R273H	c.818G>A	chr17: g.7673802G>A

Table 2 DNA-Fusions present in the SensID PanCancer cfDNA Reference Standard. HGVS = Human Genome Variation Society

Gene 1::Gene 2	Fusion point gene 1 (GRCh38)	fusion point gene 2 (GRCh38)
EML4::ALK	chr2: g.42264731 (exon 6)	chr2: g.29223528 (exon 20)
EML4::ALK	chr2: g.42325554 (exon 20)	chr2: g.29223528 (exon 20)
EML4::ALK	chr2: g.42295516 (exon 13)	chr2: g.29223528 (exon 20)
CD74::ROS1	chr5: g.150404680 (exon 6)	chr6: g.117324415 (exon 34)
CD74::ROS1	chr5: g.150404680 (exon 6)	chr6: g.117329446 (exon 32)
ETV6::NTRK3	chr12: g.11869969 (exon 5)	chr15: g.87940753 (exon 15)
FGFR3::BAIAP2L1	chr4: g.1806934 (exon 17)	chr7: g.98362432 (exon 2)
FGFR3::TACC3	chr4: g.1806934 (exon 17)	chr4: g.1739702 (exon 11)
LMNA::NTRK1	chr1: g.156130773 (exon 2)	chr1: g.156874571 (exon 10)
NACC2::NTRK2	chr9: g.136013864 (exon 4)	chr9: g.84744973 (exon 11)
NPM1::ALK	chr5: g.171391799 (exon 4)	chr2: g.29223528 (exon 20)
QKI::NTRK2	chr6: g.163563719 (exon 6)	chr9: g.84867243 (exon 14)
CCDC6::RET	chr10: g.59906122 (exon 1)	chr10: g.43116584 (exon 12)
KIF5B::RET	chr10: g.32028428 (exon 15)	chr10: g.43116584 (exon 12)
NCOA4::RET	chr10: g.46012883 (exon 8)	chr10: g.43116584 (exon 12)
EZR::ROS1	chr6: g.158770764 (exon 10)	chr6: g.117324415 (exon 34)
SLC34A2::ROS1	chr4: g.25664330 (exon 4)	chr6: g.117329446 (exons 34)
TPM3::NTRK1	chr1: g.154170400 (exon 7)	chr1: g.156874571 (exon 10)
TRIM24::NTRK2	chr7: g.138573642 (exon 12)	chr9: g.84867243 (exon 14)

Creating a Workflow Control by Spiking cfDNA into Plasma

The natural concentration of cfDNA in patient samples can vary significantly.

As a reference, concentrations from international proficiency testing programs can be used:

- Typically, **around 30 ng cfDNA per ml of plasma** are used
- Some providers use up to **80 ng/ml**.

Please note:
The actual amount available after extraction depends on the extraction efficiency of the system used.

Example: With 50 % extraction efficiency, starting with 30 ng/ml plasma would result in a yield of approximately 15 ng/ml cfDNA.

Step 1: **Preparation:**
- Briefly centrifuge the cfDNA and plasma to avoid liquid remaining in the vial caps.
- Work in a clean environment (e.g., a laminar flow hood) to prevent contamination.

Step 2: **Submitting 1X Plasma:** Pipette a defined volume of 1X plasma appropriate for your workflow requirements.
Step 3: **Adding cfDNA:** Add an appropriate amount of cfDNA (*Refer to the Certificate of Analysis (CoA) for the cfDNA concentration).*
Step 4: **Mixing:** Mix the suspension by gently pipetting up and down 10 times. Do not vortex! Briefly centrifuge again to ensure no liquid is lost.

Example:

Input parameter	• **cfDNA concentration** (according to CoA): 20.3 ng/µl • **Target concentration**: 30 ng/ml plasma • **Target volume**: 2 ml 1X plasma
Calculation result	• 30 ng/ml × 2 ml = 60 ng total cfDNA required • 60 ng ÷ 20.3 ng/µl = **2.96 µl cfDNA** → Pipette 2 ml of 1X plasma into a suitable tube, add 2.96 µl cfDNA, and mix as described.

Additional Notes:
- Adjust the volumes as needed depending on your desired cfDNA target concentration.
- Regularly validate your results, especially if you change the extraction system or the source of cfDNA.