ESR1 & PIK3CA Reference Set 1 % AF cfDNA (SID-000157) – Instructions for use

For Research Use Only

SensID Bringing Precision to Molecular Diagnostics

Every diagnostic test as well as R&D needs references and controls. SensID GmbH manufactures High Quality Reference Materials / Controls for Molecular Diagnostics.
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.	
ESR1 & PIK3CA Reference Set 1 % AF cfDNA	SID-000157	
Consisting of	**Internal No.**	**Target Quantity**
ESR1 & PIK3CA Reference Vial 1 1 % AF cfDNA	SID-000158	250 ng dsDNA
ESR1 & PIK3CA Reference Vial 2 1 % AF cfDNA	SID-000159	250 ng dsDNA
ESR1 & PIK3CA Reference Vial 3 1 % AF cfDNA	SID-000160	250 ng dsDNA
ESR1 & PIK3CA Reference Vial 4 1 % AF cfDNA	SID-000161	250 ng dsDNA
ESR1 & PIK3CA Reference Vial 5 WT 0 % AF cfDNA	SID-000162	1250 ng dsDNA
Target concentration 10 ng/µl 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

The product contains a precisely defined allele frequency as indicated on the corresponding certificate. It was designed for use in **liquid biopsy** with the **intended application:**

1. For spike-in experiments: can be spiked in Plasma (human-tech) DNA free for use as full workflow controls
2. As comparative sample for validation of processes for the verification of the gene-mutation
3. Control in workflow verification / validation
4. Validation and development of sequencing protocols and PCR protocols
5. Analyze the performance of your NGS pipeline

Protocol: ESR1 & PIK3CA Reference Set 1 % AF cfDNA

Important point before starting:

- It is recommended to centrifuge SID-000157 briefly to avoid liquid holding back in the lid of the vial!
- To avoid contaminations in the vial work in clean environment (e.g. laminar flow hood)
- Mix by pipetting up and down 10 times to obtain a homogeneous suspension. Do not vortex!
- No further purification or DNA isolation steps needed
- DNA purified from a proprietary cell line
- 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 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 particular product 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 ESR1 & PIK3CA Reference Set 1 % AF cfDNA 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. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at https://www.sens-id.com/shop/cfdna-ctdna-en/esr1-pik3ca-reference-set-1-af-cfdna/ where you can find, view, and print the SDS for each SensID GmbH products, kit component and other products.
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 a proprietary cell line, which is a B-lymphocytic, female cell line.

Equipment and Reagents to Be Supplied by User

- Pipets (adjustable)
- Sterile pipet tips with filters

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Table 1 General information about ESR1 and PIK3CA. Taken from https://www.ncbi.nlm.nih.gov/gene/2099 and https://www.ncbi.nlm.nih.gov/gene/5290

Official Symbol	**ESR1**
Official full name	estrogen receptor 1
Organism	Homo sapiens
Also known as	ER; ESR; Era; ESRA; ESTRR; NR3A1
Summary	This gene encodes an estrogen receptor and ligand-activated transcription factor. The canonical protein contains an N-terminal ligand-independent transactivation domain, a central DNA binding domain, a hinge domain, and a C-terminal ligand-dependent transactivation domain. The protein localizes to the nucleus where it may form either a homodimer or a heterodimer with estrogen receptor 2. The protein encoded by this gene regulates the transcription of many estrogen-inducible genes that play a role in growth, metabolism, sexual development, gestation, and other reproductive functions and is expressed in many non-reproductive tissues. The receptor encoded by this gene plays a key role in breast cancer, endometrial cancer, and osteoporosis. This gene is reported to have dozens of transcript variants due to the use of alternate promoters and alternative splicing, however, the full-length nature of many of these variants remain uncertain. [provided by RefSeq, Jul 2020]
Official Symbol	**PIK3CA**
Official Full Name	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit
Organism	*Homo sapiens*
Also known as	MCM; CWS5; MCAP; PI3K; CLAPO; CLOVE; MCMTC; PI3K-alpha; p110-alpha
Summary	Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of this gene has been defined on chromosome 22. [provided by RefSeq, Apr 2016]

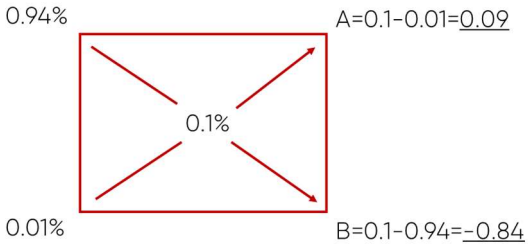
Table 2 Mutations present in the SensID ESR1 & PIK3CA 1 % AF cfDNA reference material. HGVS = Human Genome Variation Society; GRCh38 COSMIC v99, SID-000162 ESR1 & PIK3CA Reference Vial 5 exclusively contains the sequences verified as wildtype during testing.

SID-Ref	Gene	COSMIC ID	Type of mutation	Amino acid change	Localisation in Genome (GRCh38)	HGVS Nomenclature
SID-000158	ESR1	COSV52782264	Substitution	p.E380Q	6:152011697-152011697 Exon 5	c.1138G>C
	ESR1	COSV52782924	Substitution	p.Y537C	6:152098788-152098788 Exon 8	c.1610A>G
	PIK3CA	COSV55873209	Substitution	p.E545A	3:179218304..179218304 Exon 9	c.1634A>C
	PIK3CA	COSV55874020	Substitution	p.C420R	3:179210192..179210192 Exon 7	c.1258T>C
SID-000159	ESR1	COSV52782930	Substitution	p.L536P	6:152098785-152098785 Exon 8	c.1607T>C
	ESR1	COSV52783938	Substitution	p.Y537S	6:152098788-152098788 Exon 8	c.1610A>C
	PIK3CA	COSV55873227	Substitution	p.E542K	3:179218294..179218294 Exon 9	c.1624G>A
	PIK3CA	COSV55876869	Substitution	p.Q546R	3:179218307..179218307 Exon 9	c.1637A>G
SID-000160	ESR1	COSV52787207	Substitution	p.L536R	6:152098785-152098785 Exon 8	c.1607T>G
	ESR1	COSV52781024	Substitution	p.D538G	6:152098791-152098791 Exon 8	c.1613A>G
	PIK3CA	COSV55873239	Substitution	p.E545K	3:179218303..179218303 Exon 9	c.1633G>A
	PIK3CA	COSV55876499	Substitution	p.H1047Y	3:179234296..179234296 Exon 20	c.3139C>T
SID-000161	ESR1	COSV52784970	Substitution	p.S463P	6:152094402-152094402 Exon 7	c.1387T>C
	ESR1	COSV52784978	Substitution	p.Y537N	6:152098787-152098787 Exon 8	c.1609T>A
	PIK3CA	COSV55873220	Substitution	p.E545G	3:179218304..179218304 Exon 9	c.1634A>G
	PIK3CA	COSV55873195	Substitution	p.H1047R	3:179234297..179234297 Exon 20	c.3140A>G
SID-00162	ESR1	wildtype				
	PIK3CA	wildtype				

How to lower the allele frequency with wildtype

The Pearson Square is a graphical method used to determine the proportions of two solutions needed to achieve a desired concentration or mixture.

Protocol example:



1. Step 1: **Setting up the square:** Draw a square and write the initial concentration of one solution on top left corner (e.g., 0.94 % allele frequency-AF of a ESR1 mutant) and the initial concentration of the other solution on the bottom left corner, in this case wildtype (e.g., 0.01 %).
2. Step 2: **Write the required or targeted concentration** : It shall be written in the middle of the square (e.g., 0.1 % AF).
3. Step 3: **Filling in the square:** Start by subtracting the concentration of the wildtype from the target concentration, along the diagonal line resulting in A (e.g., A=0.1 %; A-0.01 % AF=0.09 %).
4. Step 4: **Balancing the square:** Subtract the initial (higher) from the desired concentration, diagonally across the square, which results in B (B=0.1 %-0.94 % AF). Disregard the negative value of B and take it as positive value (e.g., B=0.84) to ensure that the proportions of the two ingredients in the final mixture are correct.
5. Step 5: **Reading the intersection:** Divide each of the generated values (A and B) with the sum of them both to find the percent of each solution to be taken for achieving the target concentration. In the given example, to generate 0.1% AF solution of 1 %AF a ESR1 mutant, by mixing with the wildtype, one must mix 9.67% of 1 % AF ESR1 & PIK3CA mutant and 90.33 % of the wildtype.

$$\%\ \text{A for } 0.1\ \%\ \text{AF} = \frac{\text{A}}{\text{A}+\text{B}} * 100$$

$$\%\ \text{B for } 0.1\ \%\ \text{AF} = \frac{\text{B}}{\text{A}+\text{B}} * 100$$

E.g.:

$$\%\ \text{A for } 0.1\ \%\ \text{AF} = \frac{0.09}{0.09+0.84} * 100 = 9.67\ \%$$

$$\%\ \text{B for } 0.1\ \%\ \text{AF} = \frac{0.84}{0.09+0.84} * 100 = 90.33\ \%$$

IMPORTANT NOTE: ONLY POSSIBLE for mixing vials (wildtype with mutant) from within a single ESR1 & PIK3CA reference set, because only this way it is guaranteed, that the wildtype is from the same wildtype lot/background.

Cross-reactivity will depend on individual assays/technologies & conditions (e.g. temperature in PCR) used!

Users must check and correct for potential cross-reactivities if combining vials!