5-Gene-Multiplex 0.1% AF cfDNA, AKT1/BRAF/ERBB2/KRAS/PIK3CA (SID-000092) – Instructions for use

For Research Use Only

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Content

Product	Catalog No.
5-Gene-Multiplex 0.1% AF cfDNA, AKT1/BRAF/ERBB2/KRAS/PIK3CA	SID-000092

Symbols

Catalog number

Lot number


Use by


Legal manufacturer


Not for reuse


Temperature limitations


GHS07: Harmful

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
2. As comparative probe for validation of processes for the verification of the 5-Gene-Multiplex AKT1/BRAF/ERBB2/KRAS/PIK3CA 0.1% AF cfDNA mutations
3. Control in workflow verification / validation
4. Validation and development of targeted sequencing protocols (amplicon sequencing) and PCR protocols
5. Analyze the performance of your amplicon-based NGS pipeline (including capture-based assays) by comparing to freely available datasets

Protocol: 5-Gene-Multiplex 0.1% AF cfDNA

Important point before starting:

- *It is recommended to centrifuge SID-000092 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 reference cell line, GM24385*
- *The purified DNA is present in cfDNA (human) at a fragment size of 167 bp ±10%*
- *While the presence and frequency of each mutation and/or amplification in this product is evaluated during manufacture using ddPCR 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 5-Gene-Multiplex 0.1% AF cfDNA, AKT1/BRAF/ERBB2/KRAS/PIK3CA 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 www.sens-id.com, 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 the cell line GM24385, which is a B-lymphocytic, male cell line from the Personal Genome Project offered by the NIGMS Human Genetic Cell Repository (https://catalog.coriell.org/1/NIGMS).

Equipment and Reagents to Be Supplied by User

- Pipets (adjustable)[1]
- Sterile pipet tips with filters

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

Table 1 General information about intended genes. Taken from https://www.ncbi.nlm.nih.gov/gene/673, https://www.ncbi.nlm.nih.gov/gene/5290, https://www.ncbi.nlm.nih.gov/gene/3845, https://www.ncbi.nlm.nih.gov/gene/207, https://www.ncbi.nlm.nih.gov/gene/2064.

Official Symbol	**AKT1**
Official Full Name	AKT serine/threonine kinase 1
Organism	*Homo sapiens*
Also known as	AKT; PKB; RAC; CWS6; PRKBA; PKB-ALPHA; RAC-ALPHA
Summary	The serine-threonine protein kinase encoded by the AKT1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase. In the developing nervous system AKT is a critical mediator of growth factor-induced neuronal survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. Mutations in this gene have been associated with the Proteus syndrome. Multiple alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Jul 2011]
Official Symbol	**BRAF**
Official Full Name	B-Raf proto-oncogene, serine/threonine kinase
Organism	*Homo sapiens*
Also known as	NS7; B-raf; BRAF1; RAFB1; B-RAF1
Summary	This gene encodes a protein belonging to the RAF family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division, differentiation, and secretion. Mutations in this gene, most commonly the V600E mutation, are the most frequently identified cancer-causing mutations in melanoma, and have been identified in various other cancers as well, including non-Hodgkin lymphoma, colorectal cancer, thyroid carcinoma, non-small cell lung carcinoma, hairy cell leukemia and adenocarcinoma of lung. Mutations in this gene are also associated with cardiofaciocutaneous, Noonan, and Costello syndromes, which exhibit overlapping phenotypes. A pseudogene of this gene has been identified on the X chromosome. [provided by RefSeq, Aug 2017]
Official Symbol	**ERBB2**
Official Full Name	erb-b2 receptor tyrosine kinase 2
Organism	*Homo sapiens*
Also known as	NEU; NGL; HER2; TKR1; CD340; HER-2; MLN 19; HER-2/neu
Summary	This gene encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This protein has no ligand binding domain of its own and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signalling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase. Allelic variations at amino acid positions 654 and 655 of isoform a (positions 624 and 625 of isoform b) have been reported, with the most common allele, Ile654/Ile655, shown here. Amplification and/or overexpression of this gene has been reported in numerous cancers, including breast and ovarian tumors. Alternative splicing results in several additional transcript variants, some encoding different isoforms and others that have not been fully characterized. [provided by RefSeq, Jul 2008]
Official Symbol	**KRAS**
Official Full Name	KRAS proto-oncogene, GTPase
Organism	*Homo sapiens*
Also known as	NS; NS3; OES; CFC2; RALD; K-Ras; KRAS1; KRAS2; RASK2; KI-RAS; C-K-RAS; K-RAS2A; K-RAS2B; K-RAS4A; K-RAS4B; c-Ki-ras2

Summary	This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region. [provided by RefSeq, Jul 2008]
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 5-Gene-Multiplex 0.1% AF cfDNA, AKT1/BRAF/ERBB2/KRAS/PIK3CA reference material. HGVS = Human Genome Variation Society; * = GRCh38 · COSMIC v91*

Gene	Legacy Identifier	Genomic Mutation ID	Type of mutation	HGVS Nomenclature	Localisation in Genome (GRCh38)	Amino acid change
AKT1	COSM33765*	COSV62571334*	Substitution Missense	c.49G>A	14:104780214..104780214 Exon 2	p.E17K
BRAF	COSM476*	COSV56056643*	Substitution Missense	c.1799T>A	7:140753336..140753336 Exon 15	p.V600E
ERBB2	COSM20959	COSV54062409*	Insertion In frame	c.2313_2324dup	17:39724742..39724743 Exon 19	p.E770_A771insAYVM new COSMIC v91: p.Y772_A775dup
KRAS	COSM521*	COSV55497369*	Substitution Missense	c.35G>A	12:25245350..25245350 Exon 1	p.G12D
KRAS	COSM549*	COSV55502066*	Substitution Missense	c.181C>A	12:25227343..25227343 Exon 2	p.Q61K
KRAS	COSM19404*	COSV55501778*	Substitution Missense	c.436G>A	12:25225628..25225628 Exon 3	p.A146T
PIK3CA	COSM775*	COSV55873195*	Substitution Missense	c.3140A>G	3:179234297..179234297 Exon 20	p.H1047R
PIK3CA	COSM763*	COSV55873239*	Substitution Missense	c.1633G>A	3:179218303..179218303 Exon 9	p.E545K