# 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA (SID-000089) – Instructions for use

For Research Use Only

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## Content

| Product | Catalog No. |
|---|---|
| 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA | SID-000089 |

## Symbols

Catalog number

Lot number


Use by


Legal manufacturer


Not for reuse


Temperature limitations


GHS07: Harmful

## Storage

5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA should be stored at 2°C to 8°C upon arrival. DO NOT FREEZE. 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA is stable until the expiration date when stored under these conditions.

## Intended Use

5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA contains a 1-fold concentrated human-tech plasma and a precisely defined allelic frequency of 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA.

**Intended application is:**

1. DNA extraction control (e.g. instrument qualification)
2. As comparative probe for validation of processes for the verification of the EGFR-Multiplex mutations
3. Control in workflow validation
4. Validation and development of targeted sequencing protocols (Amplicon Sequencing) and PCR protocols
5. Analyze the performance of your NGS pipeline by comparing to freely available datasets

Plasma (human-tech) is human recreated DNA free plasma. It contains all relevant components of human plasma after a blood draw and plasma separation:

1. Human serum proteins in common plasma concentrations
2. Electrolytes
3. EDTA

EDTA concentration in all plasma products complies with the recommendations of ICSH (International Council Society of Haematology) and the CLSI (Clinical and Laboratory Standards Institute) for EDTA in blood collection tubes.

## Protocol: 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA

***Important point before starting:***

- ***It is recommended to centrifuge SID-000089 briefly to avoid liquid holding back in the lid of the vial!***
- ***Mix by pipetting up and down 10 times to obtain a homogeneous suspension. Do not vortex!***
- ***To avoid contaminations in the vial work in clean environment (e.g. laminar flow hood)***
- DNA purified from a reference cell line, GM24385
- The purified DNA is present in Plasma (human-tech) 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 allelic 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 5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA 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 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA is tested against predetermined specifications to ensure consistent product quality.
5-Gene-Multiplex 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA should appear as a clear to pale yellow 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/shop/plasma-human-tech-cfdna-ctdna/SID-000089 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


SensID GmbH
Schillingallee 68
D-18057 Rostock

Contact Information:
info@sens-id.com
www.sens-id.com

Orders: sales@sens-id.com
Technical assistance: tech@sens-id.com
Tailored products: tailored@sens-id.com

- 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 1% AF cfDNA in Plasma, AKT1/BRAF/ERBB2/KRAS/PIK3CA reference materials. 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 |