EGFR-Multiplex 5% AF cfDNA in Plasma (SID-000018) – Instructions for use

For Research Use Only

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Content

Product	Catalog No.
EGFR-Multiplex 5% AF cfDNA in Plasma	SID-000018

Symbols

REF	Catalog number
LOT	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 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 in a 1-fold concentrated human-tech plasma matrix. The product was designed for use in **liquid biopsy** with the **intended application:**

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 verification / validation
4. Validation and development of targeted sequencing protocols (Amplicon Sequencing) and PCR protocols
5. Analyze the performance of your NGS pipeline (including capture-based assays) 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: EGFR-Multiplex 5% AF cfDNA in Plasma

Important point before starting:

- *It is recommended to centrifuge SID-000018 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!*
- *DNA isolation from plasma required*
- *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 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 EGFR-Multiplex 5% AF cfDNA in Plasma is tested against predetermined specifications to ensure consistent product quality.
The product 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. The product contains a precisely defined allele frequency as indicated on the corresponding certificate in a 1-fold concentrated human-tech plasma matrix.

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 EGFR. Taken from https://www.ncbi.nlm.nih.gov/gene/1956.

Official Symbol	EGFR
Official Full Name	Epidermal growth receptor
Organism	*Homo sapiens*
Also known as	ERBB; HER1; mENA; ERBB1; PIG61; NISBD2
Summary	The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer. [provided by RefSeq, Jun 2016]

*Table 2 Mutations present in the SensID EGFR-Multiplex 5% AF cfDNA in Plasma 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
EGFR	COSM6252*	COSV51767289*	Substitution (missense)	c.2155G>A	7:55174014..55174014 Exon 18	p.G719S
EGFR	COSM6225*	COSV51765066*	Deletion (frameshift)	c.2236_2250del15	7:55174773..55174787 Exon 19	p.E746_A750 delELREA
EGFR	COSM6256*	COSV51774879*	Deletion (in frame)	c.2254_2277del24	7:55174791..55174814 Exon 19	p.S752_I759 delSPKANKEI
EGFR	COSM6241*	COSV51768106*	Substitution (missense)	c.2303G>T	7:55181312..55181312 Exon 20	p.S768I
EGFR	COSM20884*	COSV51850427*	Insertion (in frame)	c.2303_2304ins TGTGGCCAG	7:55181312..55181313 Exon 20	p.V769_D770insASV new COSMIC v91: p.A767_V769dup
EGFR	COSM6240*	COSV51765492*	Substitution (missense)	c.2369C>T	7:55181378..55181378 Exon 20	p.T790M
EGFR	COSM6224*	COSV51765161*	Substitution (missense)	c.2573T>G	7:55191822..55191822 Exon 21	p.L858R
EGFR	COSM6213*	COSV51766344*	Substitution (missense)	c.2582T>A	7:55191831..55191831 Exon 21	p.L861Q

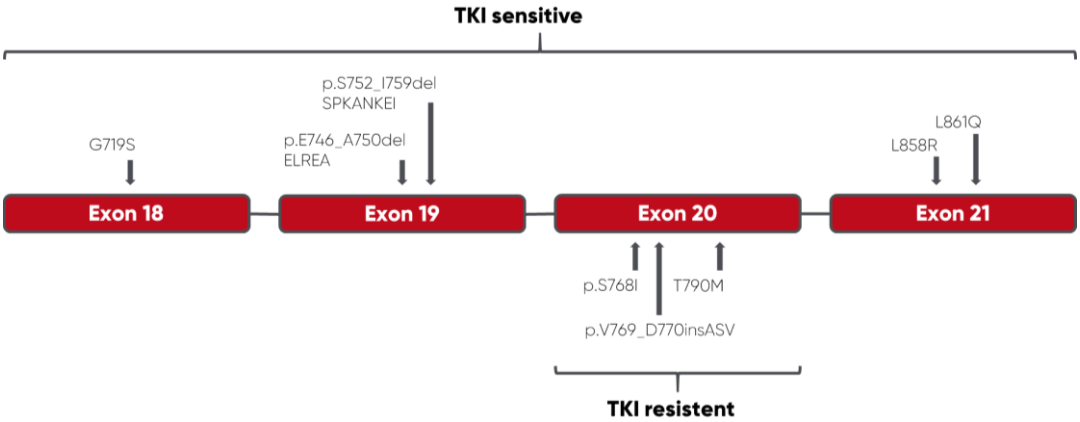

Figure 1 Schematic overview of EGFR mutations in different Exons of EGFR gene included in the product. Mutations above the scheme are associated with sensitivity to EGFR TKIs (TKIs = Tyrosine Kinase Inhibitors). Mutations listed below the scheme are associated with EGFR TKI resistance.