DPYD 19-Plex Set gDNA,
(SID-000175) – Instructions for use

For Research Use Only

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Content

Product	**Catalog No.**	
DPYD 19-Plex Set gDNA	SID-000175	
Consisting of	**Catalog No.**	**Target Quantity**
DPYD-Multiplex 0 % AF gDNA	SID-000134	**400 ng dsDNA**
DPYD 19-Plex 50 % AF gDNA	SID-000176	**400 ng dsDNA**
DPYD 19-Plex 100 % AF gDNA	SID-000177	**400 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:

1. Validation and verification of workflows, including DNA processing, amplification, sequencing, and data analysis with ready to use material
2. Development, optimization, and validation of PCR- and sequencing-based methods
3. Performance assessment and optimization of next-generation sequencing (NGS) pipelines
4. Use as a comparative sample for the verification of variant detection and overall assay performance
5. Troubleshooting of workflows and identification of assay-related issues
6. Training of laboratory personnel and competency assessment
7. Inter-laboratory comparison and harmonization of results
8. Lot-to-lot comparison and consistency testing of reagents and assay components

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

Product Background

The SensID DPYD 19-Plex Set gDNA Reference Material consists of various dihydropyrimidine dehydrogenase gene (DPYD) variants that are known to have an impact on 5-fluorouracil (5-FU) catalyzation. 5-FU is used for treatment of cancer. In patients with defective DPYD, 5-FU toxicity occurs with standard doses of 5-FU causing severe and life-threatening effects.
The SensID DPYD 19-Plex Set gDNA Reference Material consists of three vials with 0 %, 50 % and nominal 100 % mutant allele frequency. Well characterized human gDNA from proprietary cell line is used as wild-type background. For additional information see table 1 and 2. Mutations based on AMP & EMA recommendation.

Protocol: DPYD 19-Plex Set gDNA

Important point before starting:

- It is recommended to centrifuge SID-000175 briefly to avoid liquid holding back in the lid of the vial!
- To avoid contamination 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.
- While the presence and frequency of each mutation and/or amplification in this product is evaluated during manufacture using dPCR[1] assay, the detection of the allele frequency may vary depending on the assay used. Therefore, due to assay properties, there may be deviations in the observed allele frequencies compared to the values given here. Furthermore, some DPYD variants included in the DPYD 19-Plex Set gDNA are located in close physical proximity. Depending on assay design and workflow (e.g., PCR or NGS), this may affect the detection and discrimination of individual variants.

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 DPYD 19-Plex Set gDNA 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 http://www.sens-id.com/ 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 DPYD 19-Plex Set gDNA 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 a proprietary cell line, which is a female B-lymphocyte cell line.

Equipment and Reagents to Be Supplied by User

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

[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 General information about DPYD. Taken from https://www.ncbi.nlm.nih.gov/gene/1806.

Official Symbol	DPYD
Official Full Name	dihydropyrimidine dehydrogenase
Organism	*Homo sapiens*
Also known as	DHPDHASE; DHPDHase; DPD; DHP
Summary	The protein encoded by this gene is a pyrimidine catabolic enzyme and the initial and rate-limiting factor in the pathway of uracil and thymidine catabolism. Mutations in this gene result in dihydropyrimidine dehydrogenase deficiency, an error in pyrimidine metabolism associated with thymine-uraciluria and an increased risk of toxicity in cancer patients receiving 5-fluorouracil chemotherapy. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, May 2009]

Table 2 Mutations present in the SensID DPYD 19-Plex Set gDNA Reference material. HGVS = Human Genome Variation Society, based on AMP & EMA recommendation (https://www.jmdjournal.org/article/S1525-1578(24)00154-5/fulltext and https://www.ema.europa.eu/en/documents/referral/fluorouracil-and-fluorouracil-related-substances-article-31-referral-annex-iii_en.pdf-0)

Gene	dbSNP	Type of mutation	HGVS Nomenclature	Localisation in Genome (GRCh38)	Amino acid change / Synonym
DPYD	rs56038477	Substitution	1236G>A	1:97573863	p.E412=, HapB3[1)]
DPYD	rs56276561	Substitution	c.483+18G>A	1:97721492	-
DPYD	rs67376798	Substitution	c.2846A>T	1:97082391	p.D949V
DPYD	rs75017182	Substitution	c.1129–5923C>G	1:97579893	IVS10, HapB3[1)]
DPYD	rs55886062	Substitution	c.1679T>G	1:97515787	p.I560S other names: DPYD*13
DPYD	rs3918290	Substitution	c.1905+1G>A	1:97450058	IVS14, G-A, +1 other names: DPYD*2A
DPYD	rs115232898	Substitution	c.557A>G	1:97699474	p.Y186C
DPYD	rs72549309	Deletion	c.299_302del	1:97740411-97740414	p.F100fs other names: DPYD*7
DPYD	rs146356975	Substitution	c.868A>G	1:97595149	p.K290E
DPYD	rs112766203	Substitution	c.2279C>T	1:97770835	p.T760I
DPYD	rs1801266	Substitution	c.703C>T	1:97691776	p.R235W other names: DPYD*8
DPYD	rs186169810	Substitution	c.1314T>G	1:97573785	p.F438L
DPYD	rs72549304	Substitution	c.1475C>T	1:97549609	p.S492L
DPYD	rs59086055	Substitution	c.1774C>T	1:97450190	p.R592W
DPYD	rs55674432	Substitution	c.2639G>T	1:97098616	p.G880V
DPYD	rs72549310	Substitution	c.61C>T	1:97883353	p.R21*
DPYD	rs755416212	Substitution	c.704G>A	1:97691775	p.R235Q
DPYD	rs183385770	Substitution	c.1024G>A	1:97593322	p.D342N
DPYD	rs773499329	Deletion	c.2043_2058del	1:97373561_97373577	p.L682IfsTer24

1) The haplotype previously designated as “HapB3” is a decreased function allele, currently defined by two variants, linkage disequilibrium