Seraseq®
DPYD DNA Mutation Mix

Package Insert

NAME AND INTENDED USE

The Seraseq® DPYD DNA Mutation Mix is a reference material intended for use in the development, validation, and evaluation of routine performance of Next Generation Sequencing (NGS) assays that identify mutations in the DPYD gene encoding for the dihydropyrimidine dehydrogenase (DPD) enzyme, which is critical for the metabolism of fluoropyrimidine drugs such as fluorouracil/5-FU (Adrucil®), tegafur and capecitabine (Xeloda®) used in chemotherapy treatment for cancer patients. This reference material is suitable for use by clinical laboratories, research institutions, and diagnostic assay developers to ensure consistent and reliable results across different sequencing runs and laboratory conditions.

The Seraseq DPYD DNA Mutation Mix is not intended for use in patient diagnosis, treatment, or in any therapeutic procedures. It is intended for use by trained laboratory personnel proficient in NGS technologies and familiar with proper laboratory practices and quality control procedures.
For Research Use Only. Not for use in diagnostic procedures.

REAGENTS PROVIDED

Seraseq DPYD DNA Mutation Mix is a mixture of synthetic DNA constructs and genomic DNA extracted from the human cell line GM24385. It contains 39 biosynthetic DPYD mutations (not including those present in the GM24385 background) (Table 2). The product is formulated to simulate a heterozygous state for each mutation at a 50% variant allele frequency (VAF) confirmed by droplet digital PCR and measured by NGS.

Table 1. Seraseq DPYD DNA Mutation Mix

Material No.	Product	Format
0750-9502	Seraseq DPYD DNA Mutation Mix	1x 20 µL

One (1) vial, 20 µL per vial, 600 ng total mass, at a nominal concentration of 30 ng/uL is provided. The product is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer. Refer to the batch-specific Technical Product Report for exact concentration and VAF measured. Manufactured in the USA.

WARNINGS AND PRECAUTIONS

Safety and Handling Precautions

Handle Seraseq Carrier Screening DNA Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[1]. Do not pipette by mouth; do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping up with 0.5% sodium hypochlorite solution. Avoid contamination of the product when opening and closing the vials. Dispose of all specimens and materials appropriately.

STORAGE INSTRUCTIONS

Store Seraseq DPYD DNA Mutation Mix frozen at -20°C or colder. Once opened, a vial can be thawed and re-frozen up to five (5) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to five (5) or less.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq DPYD DNA Mutation Mix should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Mix by vortexing to ensure a homogeneous solution and spin briefly. Seraseq DPYD DNA Mutation Mix should be integrated into library preparation after the DNA isolation step. If a DNA shearing step is part of the workflow, Seraseq DPYD DNA Mutation Mix should be sheared and go through the target selection and library preparation in parallel with test specimens. Refer to standard assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS AND INTERPRETATION OF RESULTS

Table 2 indicates each of the mutations represented in the Seraseq DPYD Mutation Mix. While the presence and frequency of each variant in this product was confirmed during manufacture using digital PCR assays and NGS, there may be differences in observed allele frequencies due to assay characteristics. The Seraseq DPYD DNA Mutation Mix does not have assigned values for allele frequencies of the variants present. Furthermore, specific detection of variants and variant allele frequencies within the product will vary among different assays, different procedures, different lot numbers, and different laboratories.

Each laboratory must establish an assay-specific expected value and acceptance range for each variant and lot of the Mutation Mix prior to its routine use. When results for the product are outside of the established acceptance range, it may indicate unsatisfactory test performance. Possible sources of error include: deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents or change in bioinformatics pipeline parameters.

LIMITATIONS OF THE PROCEDURE

SERASEQ DPYD DNA MUTATION MIX MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

Seraseq Carrier Screening DNA Mix is not compatible with MLPA (Multiplex ligation-dependent probe amplification) assays and NGS analysis methods based only on coverage depth, since the large genomic rearrangements do not reflect copy losses or gains across the whole DNA sequence.

TEST PROCEDURES and *INTERPRETATION OF RESULTS* provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit instructions may produce unreliable results. LGC Clinical Diagnostics does not claim that others can duplicate test results exactly. Seraseq DPYD DNA Mutation Mix is not a calibrator and should not be used for assay calibration. Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

REFERENCES

1. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.

Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods. Third Edition. CLSI guideline MM09, 2023.

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Table 2: List of Mutations

Gene ID	Mutation Type	Nucleotide Change	Protein Change	dbSNP ID	GRCh37 Location	Location	Variant Length
DPYD	SNP - Nonsense	c.61C>T	p.R21*	rs72549310	Chr1: 98348909	Exon 2	1
	INDEL Microsatellite - Frameshift	c.299_302del	p.F100Sfs	rs72549309	Chr1: 98205967-98205970	Exon 4	4
	SNP - No impact on splicing	c.483+18G>A	N/A	rs56276561	Chr1: 98187048	Intron 5	1
	SNP - Missense	c.496A>G	p.M166V	rs2297595	Chr1: 98165091	Exon 6	1
	SNP - Missense	c.557A>G	p.Y186C	rs115232898	Chr1: 98165030	Exon 6	1
	SNP - Missense	c.601A>C	p.S201R	rs72549308	Chr1: 98164986	Exon 6	1
	SNP - Missense	c.632A>G	p.Y211C	rs72549307	Chr1: 98164955	Exon 6	1
	SNP - Intronic variant	c.680+139G>A	N/A	rs6668296	Chr1: 98164768	Intron 6	2
	SNP - Missense	c.703C>T	p.R235W	rs1801266	Chr1: 98157332	Exon 7	1
	SNP - Missense	c.704G>A	p.R235Q	rs755416212	Chr1: 98157331	Exon 7	1
	SNP - Missense	c.868A>G	p.K290E	rs146356975	Chr1: 98060705	Exon 9	1
	SNP - Intronic variant	c.959-51T>C	N/A	rs115349832	Chr1: 98058994	Intron 9	1
	SNP - Missense	c.1003G>T	p.V335L	rs72549306.1	Chr1: 98058899	Exon 10	1
	SNP - Missense	c.1024G>A	p.D342N	rs183385770	Chr1: 98058878	Exon 10	1
	SNP - Missense	c.1057C>T	p.R353C	rs143154602	Chr1: 98058845	Exon 10	1
	SNP - Splicing	c.1129–5923C>G	N/A	rs75017182	Chr1: 98045449	Intron 10	1
	SNP - Nonsense	c.1156G>T	p.E386*	rs78060119	Chr1: 98039499	Exon 11	1
	SNP - Synonymous	c.1236G>A	p.E412E	rs56038477	Chr1: 98039419	Exon 11	1
	SNP - Missense	c.1314T>G	p.F438L	rs186169810	Chr1: 98039341	Exon 11	1
	SNP - Missense	c.1475C>T	p.S492L	rs72549304	Chr1: 98015165	Exon 12	1

Gene ID	Mutation Type	Nucleotide Change	Protein Change	dbSNP ID	GRCh37 Location	Location	Variant Length
DPYD	SNP - Missense	c.1484A>G	p.D495G	rs111858276	Chr1: 98015156	Exon 12	1
	SNP - Missense	c.1601G>A	p.S534N	rs1801158	Chr1: 97981421	Exon 13	1
	SNP - Missense	c.1627A>G	p.I543V	rs1801159	Chr1: 97981395	Exon 13	1
	SNP - Missense	c.1679T>G	p.I560S	rs55886062	Chr1: 97981343	Exon 13	1
	SNP - Missense	c.1777G>A	p.G593R	rs145773863	Chr1: 97915743	Exon 14	1
	SNP - Synonymous	c.1896T>C	p.F632F	rs17376848	Chr1: 97915624	Exon 14	1
	SNP - Missense	c.1775G>A	p.R592Q	rs138616379	Chr1: 97915745	Exon 14	1
	INDEL - Frameshift	c.1898delC	p.P633fs	rs72549303	Chr1: 97915622-97915623	Exon 14	1
	SNP - Splice donor causing exon 14 skipping	c.1905+1G>A	p.IVS14	rs3918290	Chr1: 97915614	Intron 14	1
	SNP - Intronic variant	c.1974+75A>G	N/A	rs72728438	Chr1: 97847874	Intron 15	1
	SNP - Missense	c.2021G>A	p.G674D	rs137999090	Chr1: 97839154	Exon 16	1
	SNP - Missense	c.2194G>A	p.V732I	rs1801160	Chr1: 97770920	Exon 18	1
	SNP - Missense	c.2279C>T	p.T760I	rs112766203	Chr1: 97770835	Exon 18	1
	SNP - Missense	c.2639G>T	p.G880V	rs55674432	Chr1: 97564172	Exon 21	1
	SNP - Missense	c.2657G>A	p.R886H	rs1801267	Chr1: 97564154	Exon 21	1
	SNP - Missense	c.2846A>T	p.D949V	rs67376798	Chr1: 97547947	Exon 22	1
	SNP - Missense	c.2872A>G	p.K958E	rs141044036	Chr1: 97547921	Exon 22	1
	SNP - Missense	c.2933A>G	p.H978R	rs72547601	Chr1: 97544677	Exon 23	1
	SNP - Missense	c.2983G>T	p.V995F	rs1801268	Chr1: 97544627	Exon 23	1

NOTE: Above list does not include variants present in the GM24385 background. Target variant allele frequency is 50%. Refer to the technical Spreadsheet MKT-01017 for more details.

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