DPYD Genotyping Kit

Real-Time PCR assay

- Detects DPYD germline variants
- 26 tests per kit
- Available as RUO (US)

FOR USE WITH:

- QuantStudio™ 5
- Applied Biosystems® 7500 Fast
- Bio-Rad CFX96™
- Roche LightCycler® 480

PACKAGE CONTENTS

This product contains the following materials which is sufficient for up to 26 reactions (including all controls and samples) per detection primer mixture:

Description	Tubes
Mutation Detection Reaction Mix (2X)	3
DPYD Reaction 1-4 Detection Primer Mix 1-4 [a]	5
Homozygous Variant Control For DPYD [b]	1
Homozygous WT Control For DPYD [c]	5
Reference Calibrator [d]	5

[a] Each tube contains a distinct Detection Primer Mix for reactions 1-4 to detect its designated DPYD variants. The contents of these tubes should not be mixed.

[b] Homozygous Variant Control for DPYD (PC1) contains a mixture of synthetic DNA sequences that correspond to each variant sequence detected by this kit and the internal control.

[c] Homozygous Wild-Type (WT) Control for DPYD (PC2) contains a mixture of synthetic DNA sequences that correspond to each wild-type sequence detected by this kit and the internal control.

[d] The reference calibrator contains human genomic DNA that is used as a reference for determining relative gene expression values and making genotype calls.

HANDLING AND STORAGE

This product is shipped on frozen ice packs. The contents of the shipment should be stored at -25°C to -15°C non-frost-free freezer immediately upon receipt.

- Store all unopened components in original containers.
- Detection primer mixes should be protected from light at all times to prevent photo bleaching of the fluorescent dyes.
- Centrifuge the tubes before opening.
- The expiration date of each component is printed on each tube label. This product will maintain its performance through this date. Its performance is not guaranteed after the expiration date.

INTENDED USE:

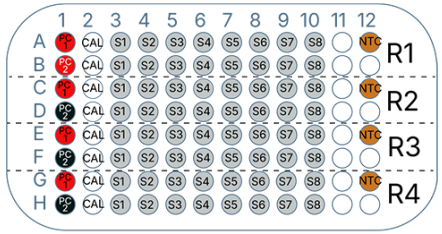
The DPYD Genotyping Kit is intended for the detection of DPYD germline variants in human genomic DNA (gDNA).
This kit is intended for research use only. Not for use in diagnostic procedures.

The DPYD variants detected by this kit are:

Reaction ID	DPYD Variant (NM_000110.4)	Legacy Name	rsID	Protein Variation
1	c.1129-5923C>G	HapB3	rs75017182	N/A
	c.1679T>G	*13	rs55886062	p. I560S
2	c.2279C>T	N/A	rs112766203	p.T760I
	c.557A>G	N/A	rs115232898	p.Y186C
3	c.868A>G	N/A	rs146356975	p.K290E
	c.2846A>T	N/A	rs67376798	p.D949V
4	c.1905+1G>A	*2A	rs3918290	N/A

PLATE SETUP

A single 96-well PCR plate accommodating eight unknown samples and one reference calibrator (CAL) run in duplicate per reaction; two positive controls (PC1 –homozygous variant control, PC2 – homozygous wild-type control) and one no-template control (NTC) run in singlet per reaction.



RESULTS INTERPRETATION

Assess Run Quality, Sample Quality and Replicate Consistency

This assay is designed to specifically amplify DPYD wild-type sequences and prevent DPYD variant sequence amplification. Thus, the homozygous variant PC (PC1), which contains synthetic gene fragments for all DPYD variant sequences targeted by the assay, should produce undetermined or very late (≥38.0) FAM and CY5 C_T values and relative quantification (RQ) values that are consistent with a homozygous variant call in every reaction. In contrast, the homozygous wild-type PC (PC2), which contains synthetic gene fragments for all DPYD wild-type sequences targeted by the assay, should produce C_T and RQ values that are consistent with a homozygous wild-type call in every reaction.
The NTC should produce undetermined or very late (≥38.5) C_T values for all reactions. For each unknown sample and calibrator replicate, ensure that the VIC C_T values for each reaction are in range. In addition, ensure consistency between all calibrator and unknown sample replicates for each reaction and detection channel (FAM, VIC, CY5). Use the tables below to assess sample quantity/quality and consistency between replicates.

For Unknown Samples and Calibrator Quantity / Quality (VIC):

Analysis Table for all instruments, not including Roche LightCycler® 480:

Internal Control C_T	Sample Quantity/Quality Status
28.5 ≤ C_T ≤ 31.0	Sufficient DNA.
C_T < 28.5	Overloaded reaction. Re-test sample with less DNA to bring VIC C_T within acceptable range
C_T > 31.0	Underloaded reaction. Re-test sample with more DNA to bring VIC C_T within acceptable range.

Analysis Table for Roche LightCycler® 480:

Internal Control C_T	Sample Quantity/Quality Status
28.0 ≤ C_T ≤ 30.5	Sufficient DNA.
C_T < 28.0	Overloaded reaction. Re-test sample with less DNA to bring VIC C_T within acceptable range
C_T > 30.5	Underloaded reaction. Re-test sample with more DNA to bring VIC C_T within acceptable range.

For Replicate Consistency of Unknown Samples and Calibrator (FAM / VIC / CY5):

All Instruments:
The C_T difference of fluorophores (FAM / VIC / CY5) between replicates for each target should be ≤ 0.65 C_T. If any fluorophore for a sample exceeds this, it should be re-tested.

Determine DPYD Genotype

Check the target-specific Relative Quantification (RQ) values calculated for each unknown sample. The DPYD genotype can be determined based on the following criteria:

In order to ensure an analogous load is present between sample and calibrator, the C_T difference between sample and calibrator VIC signals should be ≤ 1.0 C_T. If the VIC C_T difference is > 1.0 C_T, the sample should be re-run.

All Instruments:

RQ Value	DPYD Genotype
No value or 0.00 ≤ RQ ≤ 0.01	The sample is homozygous for the variant allele.
0.30 < RQ < 0.70	The sample is heterozygous for the variant allele.
RQ ≥ 0.70	The sample is homozygous for the wild-type allele.

Samples with RQ Values that Require a Re-Test

1. If the unknown sample RQ value is between 0.01 and 0.30 (0.01 < RQ ≤ 0.30), this is an invalid result. Evaluate the sample and run quality, and re-test.
2. If the unknown sample RQ value is between 0.70 to 0.85 for 2 or more variants, then this may be indicative of a low-quality DNA sample or a DNA sample that may contain PCR inhibitors. Ensure that the sample concentration is > 10 ng/µL post-extraction. The sample may need to be re-collected and re-extracted and should be re-run.

CONTACT

For more information:

+1 818 716 1070
information@entrogen.com
entrogen.com

For research use only (RUO)