

UGT1A1 Genotyping Kit

Real-Time PCR assay

- Detects UGT1A1*28 single nucleotide polymorphism in human genomic DNA
- 50 tests per kit
- Available as RUO (US) / IVD (EU)

INTENDED USE:

The UGT1A1 Genotyping Kit is intended for differentiation of the UGT1A1*1 (TA)₆ and (TA)₇ *28 polymorphism in human genomic DNA.

This kit does not identify UGT1A1 polymorphisms other than those listed above.

FOR USE WITH:

- Applied Biosystems®- 7500/7500 FAST, StepOne, StepOne Plus
- Qiagen - Rotor-Gene® Q

PACKAGE CONTENTS

This product contains the following materials:

Description	Tubes
Mutation Detection Reaction Mix (2X)	1
UGT1A1 Genotyping Primer Mix	1
UGT1A1*1 Wild-type Standard (HW)	1
UGT1A1*28 Mutant Standard (HM)	1

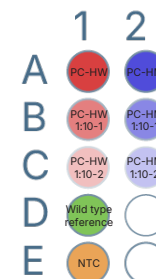
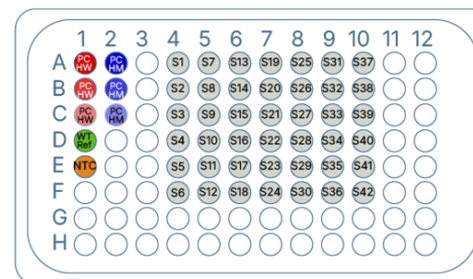
HANDLING AND STORAGE

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.
- Primer/probe mix 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.

PLATE SETUP

A single 96-well PCR plate can accommodate up to 42 unknown samples.



PRINCIPLE OF THE ASSAY

This kit is an allelic discrimination assay. This kit uses multiplexed PCR technology to amplify the promoter region of the UGT1A1 gene with oligonucleotide primers that flank the UGT1A1*1/*28 SNP. The variants are detected by hydrolysis probes labeled with two distinct fluorescent dyes. FAM labeled probes detect UGT1A1*1 (wild type) and VIC labeled probes detect UGT1A1*28 (mutant). The emitted fluorescence is collected at the end of the PCR process and then measured and recorded by a real-time PCR instrument.

Unknown samples will be classified as:

Homozygote (sample having only UGT1A1*1 or UGT1A1*28)

Heterozygote (sample having both UGT1A1*1 and UGT1A1*28)

The instruments analysis software creates a scatter plot of the UGT1A1*1 allele vs UGT1A1*28 allele. Samples are plotted due to differences in the extent of the dye fluorescent intensity after PCR amplification. To obtain tight allelic clustering and automatic allelic discrimination, sample concentrations must be the same.

ASSAY PERFORMANCE

Precision

Eight independent sample sets per sample type: mutant, wild type and heterozygous (total of 24 samples) were analyzed in triplicate by two different technicians. All samples that amplified were concordant with the known typing. No false positive signals were detected in wild-type samples.

Concordance Table

Mutation	Wild Type	Mutant	Heterozygous
UGT1A1*28	47*/48	38**/48	48/48

* 1 Wild Type sample was not typed because of loading error.

** 1 of 8 mutants in this panel had poor quality DNA and amplified 1 out of 6 times. When it amplified its typing was concordant with sequencing results. A separate mutant sample which amplified 2 out of 6 times resulted to be a UGT1A1*28/UGT1A1*36 heterozygous sample.

Limit of Detection

The limit of detection of the assay was determined using genomic DNA. 2 mutant, 2 wild type and 2 heterozygous samples were obtained at 120 ng/μl and serially diluted 1:2 down to 1.87 ng/μl. Sample were diluted in molecular grade water. Two independent reagent batches were used in this analysis. All samples that amplified were concordant with the known typing for both lots. The lower limit of detection was 3.7 ng per PCR reaction, the upper limit was 1080 ng per PCR reaction.

Note: In order to obtain automatic calls with Applied Biosystems® software it is required that all the samples in the run have the same DNA concentrations.

CONTACT

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Accuracy

The accuracy of the assay was established by testing 30 DNA samples. The mutation status of each sample was verified by Sanger sequencing. The overall concordance between the different platforms was 96.6%. Sanger sequencing was able to differentiate a UGT1A1*28/UGT1A1*36 heterozygous sample which was called mutant by the UGT1A1*28 qPCR assay. These results are summarized in the table below.

Mutation	Applied Biosystems®3130 (n=30)	Applied Biosystems®7500 (n=30)
Mutant	8	9
Heterozygous	10	9
Wildtype	12	12

Interfering Substances

EDTA did not interfere with UGT1A1 assay at 6.6 μmole/L. Hemoglobin did not interfere with UGT1A1 assay at 0.6 g/L. Heparin did not interfere with UGT1A1 assay at 41.2 units/L. The results were reproducible with two lots of UGT1A1.

For research use (RUO) in the U.S

For in vitro diagnostic use (IVD) in the European Union