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MutaREAL® UGT1A1

Real-Time-PCR Kit

For the analysis of the alleles UGT1A1*6 / *28.

Valid from 2023-07-20

REF **KF291832**
KF291896

Σ 32/96

-20 °C

IVD

CE

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Table of Contents

1 INTENDED USE

The MutaREAL® UGT1A1 Real-Time PCR Kit is a FRET-based molecular biology test for the investigation of the *6 / *28 coding mutation in the UGT1A1 gene.

2 INTRODUCTION

The enzyme UDP-glucuronosyltransferase 1-1 (gene name: UGT1A1) plays an important role in the glucuronidation pathway, in which lipophilic molecules like for example steroids, hormones or drugs are coupled with hydrophilic gluoronic acid and thereby transforms them into excretable metabolites. One of the drugs metabolised by the UDP glucuronosyltransferase 1-1 is the cytostatic Irinotecan. If the allele UGT1A1*6 or *28 is present, the glururonidation pathway is defective and Irinotecan is accumulated in the body, which can lead to severe adverse effects. [1] [2]

3 PRINCIPLE OF THE TEST

The sequence-specific MutaREAL® UGT1A1 real-time PCR kit is based on fluorescence resonance energy transfer (FRET). The assay includes two specific primers flanking the target sequence and two hybridisation probes that bind adjacent to the target sequence. One of the hybridisation probes is labelled with a donor fluorophore and, after appropriate excitation, transfers its energy to the acceptor fluorophore with which the other hybridisation probe is labelled when they are in close proximity. After the energy transfer, the acceptor dye emits light with a longer wavelength. Energy transfer can only occur when both hybridisation probes have bound to the target sequence. The amount of hybridised probe pairs and thus the fluorescence signal increases with the amount of amplified PCR product. Here, the fluorescence signal is proportional to the amount of PCR product.

Genotyping is carried out after completion of amplification by melting curve analysis. For this purpose, the temperature is slowly increased after a denaturation step and the dissociation behaviour of the hybridisation probes is recorded while continuously measuring the fluorescence. One of the hybridisation probes binds to a part of the target sequence that is present in the wild type and the mutation. The second hybridisation probe spans the mutation site. As the temperature rises, the mismatched and thus less stable probes dissociate first and the fluorescence decreases. The perfectly paired hybridisation probes dissociate later due to their higher binding energy and thus the fluorescence signal decreases only at a higher temperature.

4 PACKAGE CONTENTS

The components supplied are sufficient for the preparation of 32 (KF291832) or 96 (KF291896) reactions.

Table 1: Components of the MutaREAL® UGT1A1 Real-Time-PCR Kit .

Label	Lid Colour	Content	
		32	96
Enzyme mix	blue	1 x 438 µl	3 x 438 µl
Detection mix	yellow	1 x 368 µl	3 x 368 µl
Positive control 1 (UGT1A1*6)	red	1 x 15 µl	1 x 45 µl
Positive control 2 (UGT1A1*28)	orange	1 x 15 µl	1 x 45 µl
Negative control	green	1 x 150 µl	1 x 150 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA extraction kit (e.g. MutaCLEAN® Universal RNA/DNA, KG1038)
- Roche LightCycler® 1.5, 2.0 or 480 II real-time PCR system
  * CE conformity only exists if one of the mentioned devices is used.
- Roche LightCycler® capillaries
- Roche LightCycler® Cooling Block
- Sterile reaction tubes
- Calibrated pipettes (variable volumes) and sterile disposable tips with filter
- Optional: Liquid handling system for automation

6 TRANSPORT, STORAGE AND STABILITY

The MutaREAL® UGT1A1 real-time PCR kit is transported frozen on dry ice or cool packs. All components are to be stored protected from light at a minimum of -20 °C immediately after receipt. Avoid multiple freeze-thaw cycles (make aliqouts if necessary). Do not use after the expiry date indicated on the package.

Be sure to protect the detection mixes from direct sunlight during the entire test procedure.

7 WARNINGS AND PRECAUTIONS

Read the instructions for use carefully before using the product.

- All samples must be considered potentially infectious and/or biohazardous and all items that come into contact with the specimens must be considered potentially contaminated.
- Real-time PCR must be performed in laboratories suitable for this purpose and by specially trained personnel.
- The assay must always be carried out according to the instructions supplied with the kit.
- Areas for sample preparation and preparation of the PCR master mix should be strictly separated.
- Pipettes, tubes and other working materials must not circulate from one area to the other.
- Always use pipette tips with filters.
- Always wear powder-free disposable gloves when using the kit
- Clean pipettes and work surfaces regularly with suitable decontamination solution (no ethanol-containing agents).
- Contamination of eluates and kit components with microbes or nucleases (RNAs and DNAses) should be avoided.
- Positive and potentially positive material must be kept separate from all other kit components at all times.
- Do not open reaction tubes/plates after amplification in order to avoid contamination.
- In accordance with guidelines or requirements of local, state or federal regulations or authorised organisations, additional controls may be tested.
- Do not autoclave reaction tubes after PCR as this will not degrade the amplified nucleic acid and risks contaminating the laboratory area.
- Dispose of samples and test waste according to your local safety regulations.
- Refrigerate all PCR reagents while working.
- The purity (A260/A280) of the genomic DNA should be between 1.8 and 2.0.

8 SAMPLE MATERIAL

Starting material for the MutaREAL® UGT1A1 real-time PCR kit is genomic DNA isolated from clinical samples (blood) using a suitable extraction kit.

9 REAL-TIME-PCR

9.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-PCR familiarise yourself with the Real-Time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run the Positive Control and the Negative Control should be included.
- Before each use, all reagents must be gently thawed, thoroughly mixed (do not vortex) and briefly centrifuged.
- Protect the detection mix from exposure to light.
- We recommend always cooling the reagents and the preparation in a cooling block (+4 to +8 °C) or on ice while working.

9.2 Procedure

For amplification, one capillary / reaction tube is needed per sample plus two additional capillaries / reaction tubes for the positive controls and one capillary / reaction tube for the negative control. The following tables show the volumes to be pipetted per sample. For the analysis it is recommended to prepare a master mix for the number of samples (incl. negative and positive controls) (N) plus 10 % to compensate for inaccuracies. The master mix is pipetted as described in table 2:

Master mix - UGT1A1

Table 2: Preparation of master mix - UGT1A1

Reagent	Volume per 25 µl - reaction mix	Master mix volume
Detection mix (**yellow**)	10.5 µl	10.5 µl * (N + (N * 0.1))
Enzyme mix (**blue**)	12.5 µl	12.5 µl * (N + (N * 0.1))

- Mix the Master Mix carefully by pipetting up and down or by inverting and centrifuge briefly.
- Add **23 µl** of the Master Mix to each capillary / reaction tube.
- For the negative control add **2 µl** of the supplied negative control (**green**).

- For the positive control 1 add **2 µl** of the supplied positive control 1 (**red**).
- For the positive control 2 add **2 µl** of the supplied positive control 2 (**orange**).
- For each sample to be analysed, add **2 µl** of the extracted genomic DNA to the corresponding capillary / reaction tube.

For LigthCycler® 1.5 / 2.0: Close the capillaries with the lids, transfer them into the LightCycler® carousel and centrifuge them in the LightCycler® centrifuge (if a table-top centrifuge is used, centrifuge the capillaries in the inserts of the cooling block at 3000 rpm for 15 s). Then transfer the carousel to the LightCycler® device and start the PCR programme described in 9.3.

For LightCycler® 480 II: Close the corresponding reaction tubes (with optical foil) and centrifuge briefly. Then transfer the reaction tubes to the LightCycler® 480 II and start the PCR program described in 9.3.

9.3 Instrument settings

For the Real-Time-PCR use the thermal profile shown in table 3 for LightCycler® 1.5 and 2.0 devices or the therml profile shown in table 4 for LightCycler® 480 II devices.

Table 3: Real-Time-PCR thermal profile for LightCycler® 1.5 and 2.0

Description	Time	Temperature	Heating rate	Cycles	Acquisition
Initial Denaturation	120 s	94 °C	max.	1	none
Denaturation	10 s	94 °C	max.	45	none
Primer annealing	25 s	55 °C	max.		**single**
Elongation	25 s	72 °C	max.		none
Melting curve	20 s	95 °C	max.	1	none
	20 s	37 °C	max.	1	none
	0 s	80 °C	0.2 °C/s	1	**constant**
Cooling	30 s	40 °C	max.	1	-

Table 4: Real-Time-PCR thermal profile for LightCycler® 480 II

Description	Time	Temperature	Heating rate	Cycles	Acquisition
Initial Denaturation	120 s	94 °C	max.	1	none
Denaturation	10 s	94 °C	max.	45	none
Primer annealing	25 s	55 °C	max.		**single**
Elongation	25 s	72 °C	max.		none
Melting curve	60 s	95 °C	max.	1	none
	30 s	60 °C	max.	1	none
	30 s	50 °C	max.	1	none
	30 s	40 °C	max.	1	none
	30 s	30 °C	max.	1	none
	0 s	80 °C	**0.05 °C/s** *	1	**constant**
Cooling	30 s	40 °C	max.	1	-

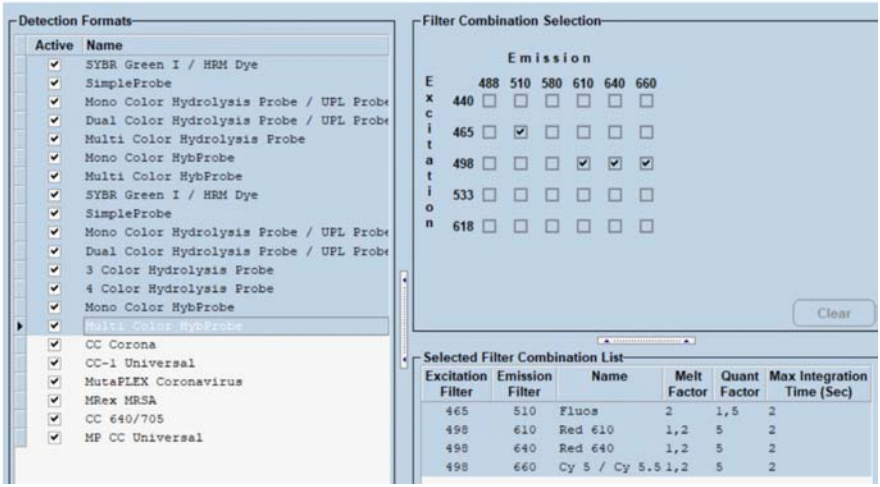
*** 6 Acquisitions per °C.**

10 DATA ANALYSIS

Add an analysis of the type „genotyping“ for the evaluation of the melting curves. This forms the derivation of the fluorescence curve. The detection wavelength is 640 nm for UGT1A1*6 and 705 nm for UGT1A1*28.

For the Roche **LightCycler® 2.0**, a Color Compensation (MutaREAL® CC universal - KF29-4-CC) is required.

For the Roche **LightCycler® 480 II** the detection format „***Multi Color HybProbe***“ is required. Otherwise, the colour compensation (MutaREAL® CC universal - KF29-4-CC) must be used.



UGT1A1*6 (640 nm)

Temperature wild type allele: 57.0 °C (+/-2 °C)

Temperature mutation allele: 62.5 °C (+/-2 °C)

The following graph shows the typical results for the possible genotypes on a LightCycler® 2.0 instrument: **light blue curve** - negative control, **pink curve** - homozygous wildtype, **black curve** - heterozygous mutant, **red curve** - homozygous mutant.

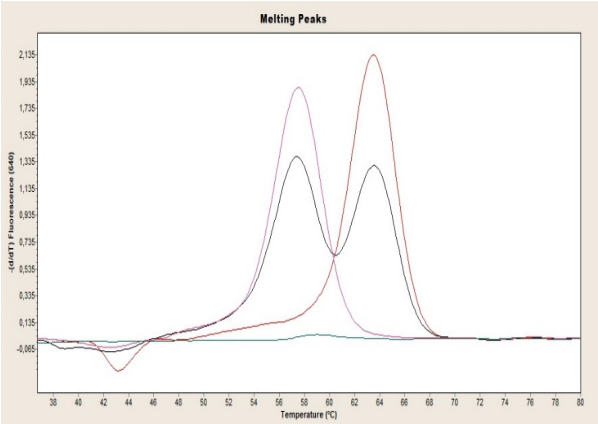


Fig. 1: Evaluation of UGT1A1*6 on a LightCycler® 2.0.

The following graph shows the typical results for the possible genotypes on a LightCycler® 480 instrument: **red curve** - negative control, **purple curve** - homozygous mutant, **green curve** - heterozygous mutant, **light blue curve** - homozygous wild type.

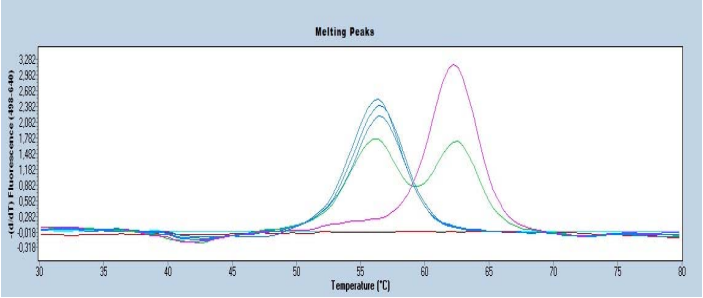


Fig. 2: Evaluation of UGT1A1*6 on a LightCycler® 480 II.

The positive control 1 (**red**) provided contains a template that is **heterozygous** for the allele UGT1A1*6.

UGT1A1*28 (705 nm)

Temperature wild type allele: 41.0 °C (+/-2 °C)

Temperature mutation allele: 47.5 °C (+/-2 °C)

The following graph shows the typical results for the possible genotypes on a LightCycler® 2.0 instrument: **light blue curve** - negative control, **pink curve** - homozygous wildtype, **green curve** - heterozygous mutant, **purple curve** - homozygous mutant.

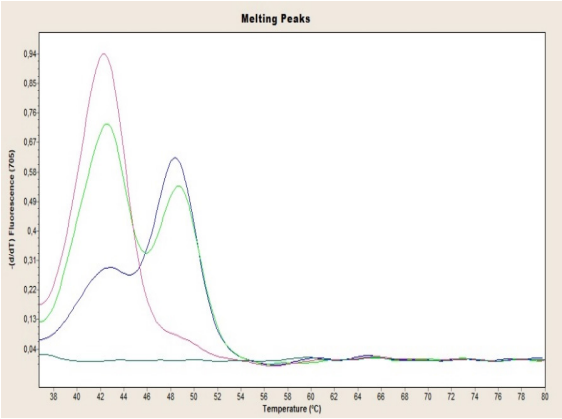


Fig. 3: Evaluation of UGT1A1*28 on a LightCycler® 2.0.

The following graph shows the typical results for the possible genotypes on a LightCycler® 480 instrument: **red curve** - negative control, **purple curve** - homozygous mutant, **green curve** - heterozygous mutant, **light blue curve** - homozygous wild type.

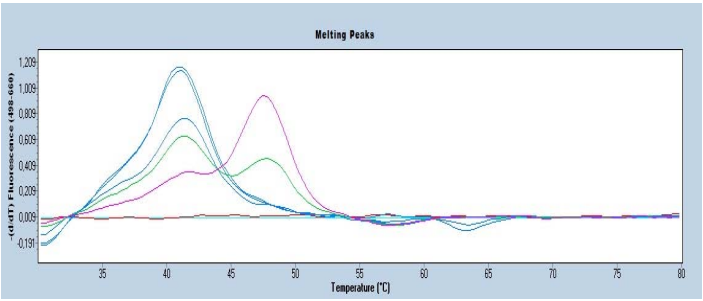


Fig. 4: Evaluation of UGT1A1*28 on a LightCycler® 480 II.

The positive control 2 (**orange**) provided contains a template that is **heterozygous** for the allele UGT1A1*28.

11 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-PCR. If you have further questions, please do not hesitate to contact our scientists on info@immundiagnostik.com.

No or weak fluorescence in the positive control or samples.

Check the PCR programme of the real-time PCR system and repeat the analysis with the corrected protocol.

Detection mix has been subjected to more than two freeze cycles or has been stored at 2-8 °C for more than four days. Repeat the analysis with a fresh aliquot or new detection mix.

The quality of the starting DNA is not sufficient. Use freshly extracted DNA and determine the concentration/purity before use.

The detection mix was not protected from light exposure. Repeat the analysis with a fresh aliquot or new PCR reagents.

12 LIMITATIONS OF THE METHOD

The result is provided to the treating physician as supporting material and should never be used exclusively for diagnosis or treatment recommendations. The diagnosis as well as the treatment decisions to be taken remain the full responsibility of the physician.

The accuracy of genetic tests is not 100 %. However, it has been found to be over 98 % accurate based on validation data for this test. Furthermore, genetic test results must be considered in the context of the patient's clinical representation and known familial risks in the patient's environment.

The test analyses only a selection of markers. Therefore, a negative test result for a patient does not completely exclude a risk of any kind.

13 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic acid	REF	Catalog number
PCR	Polymerase chain reaction	→REF	To be used with
ENZYME MIX	Enzyme mix	Σ	Contains sufficient for <n> test
DETECTION MIX	Detection mix		Upper limit of temperature
CONTROL 1 +	Positive control 1		Manufacturer
CONTROL 2 +	Positive control 2	LOT	Lot number
CONTROL –	Negative control		Consult instructions for use
IVD	*In vitro* diagnostic medical device	CONTENT	Content
	Use by YYYY-MM-DD		

14 LITERATURE

1. Takano et al., Pharmgenomics Pers Med. 2017; 10: 61–68
2. https://www.ncbi.nlm.nih.gov/books/NBK294473/