

# gb GENETIC Gilbert

Cat. No.: 3216-025  
3216-050



generi biotech

## CLINICAL IMPLICATIONS

The gb GENETIC Gilbert diagnostic kit is used to detect changes in the number of TA repetitions in the promoter of the UGT1A1 gene. This gene encodes the enzyme UDP-glucuronosyltransferase, which is responsible for the conjugation phase of biotransformation of bilirubin. The unmutated gene variant contains 6 TA repeats in the promoter, the mutated variant most frequently 7 TA repeats (haplotype designation UGT1A1\*28). Less frequently, there may be variations with 5 TA repeats (UGT1A1\*36) or 8 (UGT1A1\*37).

Homozygous carriers of genotype with TA insertion (UGT1A1\*28/\*28) are affected by so called Gilbert's syndrome, which is manifested by mild chronic unconjugated hyperbilirubinemia. This is caused by decreased glucuronidation activity of the enzyme UDP-glucuronosyltransferase to 20–30%. A number of genetic variants can lead to a phenotype of Gilbert's Syndrome, however polymorphism UGT1A1 \* 28 is the most common cause in the Caucasian and Afro-American populations. Asians with Gilbert's syndrome are often affected by polymorphism UGT1A1\*6, haplotypes UGT1A1\*36 and UGT1A1\*37 are found mainly among Afro-Americans.

Gilbert's syndrome is a benign disease not requiring treatment; nevertheless the diagnosis is appropriate in excluding severe hepatic impairment associated with hyperbilirubinemia. Gilbert's syndrome is represented in the Indo-European population with a frequency of 3–15%.

People with genotype UGT1A1\*1/\*28 (heterozygotes) are carriers of Gilbert's syndrome. The syndrome occurs, also in cases of compound heterozygotes, which carries the insertion sequence of UGT1A1 TA on one allele of the gene and on the second another mutation (UGT1A1\*28/other mutation). There are about 2% of such patients with Gilbert's Syndrome.

Detection of this mutation is also useful before starting treatment with medications that are metabolized by UDP-glucuronosyltransferase (e.g. chemotherapeutic irinotecan), its limited biotransformation can lead to the development of toxic effects (leukopenia, diarrhea, hematological toxicity). Genotyping enables timely dose reduction or selection of alternative therapy.

## SYMBOLS USED IN LABELS

	Lot Number
	Expiration Date
	Manufacturer
	Amount of Tests
	Contents
	Use for <i>in vitro</i> diagnostics
	Storage Conditions

## ADDITIONAL PRODUCTS

gb GENETIC HFE

Cat. No.: 3208

## REFERENCE

Kit gb GENETIC Gilbert was evaluated on the following real-time PCR cyclers:

- Rotor-Gene 3000/6000/Q (Corbett Research, Qiagen)
- CFX96/CFX96Touch (Bio-Rad)
- ABI 7500/7500 Fast (Applied Biosystems)
- AriaMx/Stratagene Mx3000P/Mx3005P (Agilent Technologies)
- MIC (Bio Molecular Systems)

See user instructions for the particular instrument before using the kit in combination with other types of real-time PCR cyclers.

For further information please contact us at the e-mail address [info@generi-biotech.com](mailto:info@generi-biotech.com) or phone number +420 495 056 314. More information is also available on our website: [www.generi-biotech.com](http://www.generi-biotech.com).

## TROUBLESHOOTING

Results can be considered valid only if the instructions written in the enclosed user guide are strictly kept. Positive Control signal has to be detected in appropriate fluorescent channel, Negative Control signal should not be detected in any channel. In case of incorrect results of control samples please check again the following:

- Reagents expiration date
- Storage conditions
- Real-time PCR cyclers and pipette settings



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## WARNINGS AND SAFETY PRECAUTIONS

### Shipping and Storage Conditions

The Real-time PCR kit is shipped on ice and should be stored immediately upon receipt at a temperature lower than **-20 °C** in a constant temperature freezer and in darkness (assay is photosensitive). When stored under these conditions and handled correctly, this product can be used at least until the expiration date (see the label on the box and the vial) without any reduction of performance. If frequent usage is supposed (more than **5 freeze-thaw cycles**) we recommend preparing appropriate aliquots.

### Safety Precautions

- For professional use only.
- Always wear gloves while handling these reagents and avoid any skin contact. In case of contact, immediately flush eyes or skin with copious amounts of water.
- Eating, drinking and smoking are forbidden during the manipulation with qPCR reagents.
- Use only automatic micropipettes.

### Technical Precautions

- Before starting, read the instructions completely and carefully (use the valid version). Be sure that all information written there is understood.
- Inappropriate handling or changes in working procedure may affect the results. Hence, strictly obey the pipetting volumes, incubation time and temperature according to the instructions.
- Obey the expiration date, do not use expired reagents.
- In case of damage to the package please contact the producer immediately. Do not use damaged components but keep them safe for complaint related issues.
- Use Deionized Water (enclosed).
- Prepared or used reagents have to be treated according to national safety guidelines or regulations.
- Use calibrated pipettes and instruments.

### Preventing Contamination

Please follow these recommendations:

- Wear a clean lab coat and disposable gloves (not previously worn during sample preparation) when preparing mixtures for PCR.
- Change gloves whenever you suspect they are contaminated.
- Maintain separate areas, specific equipment, and supplies for sample preparation and PCR setup.
- Never open amplified PCR products in the PCR setup area.
- Keep reaction mixtures and their components capped as much as possible.
- Use disposable pipette tips containing filters to minimize cross-contamination.

## PURPOSE AND CONTENTS

### Purpose of the Product

The kit is intended for detection polymorphism TA (allele 7TA) in gene *UGT1A1* in human genomic DNA by real-time PCR method. Use for *in vitro* diagnostics.

### Principle of Analysis

The kit is based on polymerase chain reaction (PCR) with analysis of melting curves using fluorescently labelled probe. The kit contains all components necessary for analysis.

### Contents

**	Description	Amount	Conc.	Purpose
●	Assay CQ UGT1A1 6TA/7TA	1× 0.4 ml * 2× 0.4 ml	1.25×	Detection Assay 25 rxn Detection Assay 50 rxn
○	Deionized Water	1× 1.0 ml		Negative Control
●	Standard WT UGT1A1 6TA	1× 0.2 ml	1×10 <sup>4</sup> cop/μl	Positive Control
●	Standard MUT UGT1A1 7TA	1× 0.2 ml	1×10 <sup>4</sup> cop/μl	Positive Control
●	Standard HET UGT1A1 6TA/7TA	1× 0.2 ml	1×10 <sup>4</sup> cop/μl	Positive Control

\* 25 rxn – the number of reactions is calculated for a volume of 20 μl / PCR reaction

\*\* Lid colour

### Assay CQ (contact quenching)

Assay CQ UGT1A1 qPCR is a mixture of amplification primers, fluorescently labelled probe, buffer, nucleotides and polymerase.

DNA is amplified by primers. The mixture of reagents also contains DNA probe. In the hybridized state the fluorescent probe is silenced, during melting curve analysis probe-template duplex dissociated and increase of the fluorescence signal. This change shows in the graph dF/dT a peak with a maximum at a temperature when exactly half of the duplex has dissociated (melting temperature – T<sub>m</sub>). Duplex probes and completely complementary template (perfect match) has a higher melting point than the duplex probe and a template comprising a mutation (mismatch). Based on analysis of melting temperature of the duplex probe-template is then genotype determined.

### Standard UGT1A1 (Positive Control)

The kit contains Positive Controls for each of genotypes:

- Standard WT 6TA (wild-type homozygote)
- Standard MUT 7TA (mutated homozygote)
- Standard HET 6TA/7TA (heterozygote)

At least one Positive Control for each genotype should be added in each analysis. A positive result of the standard signifies correct analysis (primers and probe work properly). In the case of a negative result the analysis is incorrect and it is necessary to reanalyze all the samples included. Be aware of contamination of other kit components by Positive Control because it could lead to false positivity. It is recommended to manipulate with Positive Control (Standard) in an area separated from the place where the PCR mixtures are prepared. Before adding Positive Control it is advised to close tubes containing samples and Negative Control. It prevents other samples / Negative Control cross-contamination during Positive Control addition.

### Deionized Water (Negative Control)

For exclusion of contamination each analysis should involve Negative Control reaction for each detection Assay. Add Deionized Water as a template instead of sample. Negative results signify absence of contamination during qPCR mixture preparation. If the result of Negative Control analysis is positive, such analysis is incorrect and unreliable and it is necessary to reanalyze all samples included. Prior to new analysis try to find and eliminate potential causes of contamination.

### Additional reagents and instruments

- Kit or reagents for DNA isolation
- Tubes for reagents mixing, real-time PCR tubes, strips or plates compatible with real-time PCR cyclers
- Adjustable automatic micropipettes (10–1000 μl)

- Sterile disposable pipette tips containing filters
- Vortex and laboratory centrifuge
- Real-time PCR cycler with software

The instructions for real-time cycler settings are available on our GENERI BIOTECH website ([Real-time PCR cycler Settings](#)).

## WORKING PROCEDURE

### Reagent Preparation

- Reagent concentrations are ready for direct use.
- Thaw all reagents before use.
- During the working procedure keep reagents, which you are not using at that moment, in the fridge and in darkness.
- Vortex all solutions and spin down shortly prior to use.
- Always prepare an exact amount of reagents sufficient for each analysis.
- If there is more frequent usage than 5 freeze thaw cycles we recommend preparing appropriate aliquots.

### Sample Preparation

The kit is intended for detection of mutation in human genomic DNA. Total amount of genomic DNA in one reaction should be in a range 10–400 ng, which corresponds to the sample concentration of 2.5–100 ng/μl.

### qPCR Mixture Preparation

Thaw Assay qPCR, vortex properly and spin down shortly prior to use. Total volume of qPCR reaction is 20 μl. Distribute **16 μl** of Assay qPCR (CQ UGT1A1) into reaction tubes. Add **4 μl** of template (DNA sample or Standard UGT1A1). In the case of lower sample volume fill the reaction up with Deionized Water to a total volume of 20 μl. Use 4 μl of Deionized Water as Negative Control.

Vortex gently and spin down shortly the final qPCR mixture.

It is better to run the qPCR analysis immediately after preparation. It is possible to store reactions in the fridge before running analysis but not for longer than one hour.

### Thermal Profile Settings

Set up the cycling conditions in the real-time PCR cycler. The recommended cycling is as follows:

<b>amplification:</b>		
initial denaturation	95 °C	3 min
<b>50 cycles of:</b>		
denaturation	95 °C	10 s
annealing	60 °C	10 s* (fluorescence acquisition)
elongation	72 °C	20 s

<b>melting analysis:</b>		
denaturation	95 °C	1 min
hybridization	35 °C	3 min
melting	35–70 °C	1 °C / 5 s** (fluorescence acquisition)

\* for cycler **ABI 7500/7500 Fast** (Applied Biosystems) set the time of annealing to 30 s

\*\* for cycler **CFX 96™** (BioRad) it is necessary to adjust the sensitivity of melting analysis for 0.5 °C / 5 s

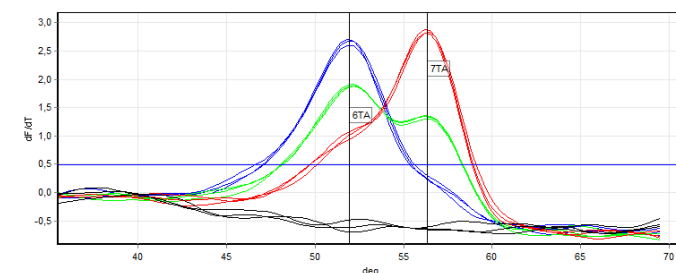
\*\* for cycler **AriaMx** (Agilent Technologies) set the melting temperature to 40–70 °C

The fluorescence is acquired during annealing phase (but it's not necessary, because the fluorescence signal does not change during amplification phase) and during melting analysis in channel for FAM/Sybr (excitation wavelength 495 nm, emission wavelength 520 nm). For cycler **Rotor-Gene** is necessary to calibrate the sensitivity of the cycler before starting a run. Set the gain so the fluorescence of the samples at 60 °C will be in the range from 30 to 60 RFU.

The instructions for real-time cycler settings are available on <http://www.generi-biotech.com/manuals-for-setting-cyclers> ([Real-time PCR cycler Settings](#)).

## RESULTS INTERPRETATION

- For evaluation of the presence a mutation in a test sample, use your real-time PCR software for analysis of melting curves.
- The result from analysis is a curve with one or more peaks in the area of T<sub>m</sub>. The obtained shape of curves and melting temperature must be compared with curves of positive controls.
- Melting curve analysis graph usually displays the decrease of the fluorescence in dependence of temperature increase (-dF/dT). In this view curves exhibit peaks in negative orientation. If the software does not analyze negative peaks, it is necessary to convert the curves to display an increase of the fluorescence in dependence of temperature increase (dF/dT). For this operation is used in software for cycler Rotor-Gene a function Flip sign of dF/dT. This function is necessary turn off for successful analysis.
- For the correct interpretation of results, it is necessary to analyse the positive control. First shown only a sample of all positive controls (WT, MUT and HET) and a negative control.
- Set the threshold to the position when is correctly determine genotypes (see Fig. 1).
- Determine T<sub>m</sub> of standards 6TA and 7TA. Melting temperature of template 7TA is in cycler Rotor-Gene 3000 about 56.5 °C and for template 6TA about 52° C. Among cyclers T<sub>m</sub> may vary.
- Check the correctness of determination of HET standards, the curve should show two balanced peaks in the area of T<sub>m</sub> for genotypes 6TA and 7TA.
- For some cyclers, a small peak in the 6TA region may occur for the MUT 7TA standard. In this case, the samples cannot be evaluated automatically based on the obtained T<sub>m</sub>. Samples must be evaluated by comparing the shape of the melting curve with the positive controls.
- Analyze the DNA sample. With the recommended quantity of DNA in the analysis should peaks exceed the adjusted threshold and the analysis can be evaluated automatically by using the modul Genotypes. Otherwise, it is necessary to analyze samples with the reduced threshold value, then it is necessary visually inspect the waveform of curves and determine the similarity with the relevant standards. Visual inspection of the curves must be done.
- In case of allele 5TA is peak T<sub>m</sub> about 3 °C lower compared with the template 6TA. Allele 8TA cannot be differentiated by T<sub>m</sub> from 6TA allele.
- If the results of all positive and negative controls are correct, however in the test sample is measured weak signal, it is necessary to analyze the new isolate of DNA. In case of incorrect results for control samples, repeat PCR analysis of all samples in this series.



**Fig. 1:** Melting curves UGT1A1 in Rotor-Gene 3000 software  
WT (6TA) blue, MUT (7TA) red, HET (6TA/7TA) green, NTC black.  
Threshold displayed as a blue line.