Instruction for Use

Dr. Schröders
LaktaseCheck real time PCR Kit

Test for the analysis of the C/T polymorphism at position -13910 within the regulatory region of the lactase gen in man.

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- 18°C

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1 Intended Use

Dr. Schröders LaktaseCheck real time PCR Kit detects the C/T (-13910) polymorphism within the regulatory region of the lactase gene using real time detection in the capillary system of the LightCycler® 1.5 or 2.0 (Roche Diagnostics).

2 Introduction

About 15 to 20 % of the population in Central Europe suffer from a primary lactose intolerance. This is mainly due to a C/T polymorphism at position -13910 within the regulatory region of the lactase gene. Individuals carrying C instead of T on both alleles develop a manifestation of a lactase deficiency with aging (often around the age of 20). In the absence of lactase, lactose remains uncleaved and passes intact into the colon, where the lactose is fermented by the bacteria in the large intestine, causing the typical symptoms of lactose intolerance: abdominal pain, nausea, diarrhea and flatulence.

3 Principle of the Test

Dr. Schröders LaktaseCheck real time PCR Kit contains specific primers and hybridization probes for the analysis of the C/T polymorphism at position -13910 within the regulatory region of the lactase gene.
The specific primers allow the amplification of the target sequence.
The identification of different genotypes in each single sample is characterized by melting curve analysis determining the binding affinity of the hybridization probe to the DNA template. The fluorescence is measured in the F2 channel (LightCycler® 1.5 or 2.0).

4 Package Contents

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of Dr. Schröders LaktaseCheck real time PCR Kit.

Label	Lid Colour	Content
Enzyme	blue	3 x 4 µl
Enzyme Buffer	blue	3 x 60 µl
Reaction Mix	yellow	2 x 700 µl
Positive Control Genotype T/T (tolerant)	red	1 x 50 µl
Positive Control Genotype C/C (intolerant)	red	1 x 50 µl
Negative Control	green	1 x 50 µl
$MgCl_2$	colourless	1 x 160 µl

5 Equipment and Reagents to be Supplied by User

- DNA isolation kit (e.g. NukEx Pure RNA/DNA, gerbion Cat. No. G05004)
- PCR grade water
- Sterile microtubes
- Pipets (adjustable volume)
- Sterile pipet tips with filter
- Table centrifuge
- Vortexer
- Real time PCR instrument LightCycler® 1.5 or 2.0
- LightCycler® Capillaries
- Optional: Liquid handling system for automation

6 Transport, Storage and Stability

Dr. Schröders LaktaseCheck real time PCR Kit is shipped on dry ice or cool packs. All components must be stored at maximum -18°C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. The reagents should not be thawed and frozen more than 2 times. If so, the Primer-Probe Mix and Positive Controls have to be aliquoted. Protect kit components from direct sunlight during the complete test run.

7 Important Notes

- Dr. Schröders LaktaseCheck real time PCR Kit must be performed by qualified personnel only.
- Good Laboratory Practice (GLP) has to be applied.
- Clinical samples must always be regarded as potentially infectious material and all equipment used has to be treated as potentially contaminated.

8 General Precautions

- Stick to the protocol described in the Instruction for Use.
- Set up different laboratory areas for the preparation of samples and for the set up of the PCR in order to avoid contaminations.
- Pipettes, tubes and other materials must not circulate between those different laboratory areas.
- Always use filter tips.
- Regulary decontaminate equipment and benches with ethanol-free decontaminant.
- Do not combine Dr. Schröders LaktaseCheck real time PCR Kit components of different lot numbers.

9 Sample Material

Sample material to be detected is genomic DNA, isolated from a buccal (cheek) swab or from EDTA blood.

10 Sample Preparation

For the procedure of the Dr. Schröders LaktaseCheck real time PCR DNA isolated by adequate methods is needed.
Commercially available kits for DNA isolation such as the following are recommended, e.g.:
• NukEx Pure RNA/DNA Kit (gerbion, Cat. No. G05004)

Further information about DNA isolation is to be found in the extraction kit manual or from the extraction kit manufacturer‘s technical service.
Important: In addition to the samples always run a ‚water control‘ in your extraction. Treat this water control analogous to a sample. The water control will show you possible contaminations.
If the real time PCR is not performed immediately, store extracted DNA due to the declaration of the manufacturer of the DNA isolation kit.

11 Real time PCR

11.1 Important Points before Starting:

- Please pay attention to the chapter 7 ‚Important Notes'.
- 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 at least one Positive Control Genotype T/T and Genotype C/C as well as one Negative Control should be included.
- Before each use, all reagents - except the Enzyme - should be thawed completely at room temperature, thoroughly mixed (do NOT vortex the Reaction Mix but mix by pipetting up and down repeatedly), and centrifuged very briefly.

11.2 Procedure

The Master Mix contains all of the components needed for PCR except the sample. Prepare a volume of Master Mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Important: Before pipetting the Master Mix, pipet one vial of Enzyme Buffer into one vial of Enzyme to receive the ready to use Enzyme Mix.

Table 2: Preparation of the Master Mix

Volume per Reaction	Volume Master Mix
14.4 µl Reaction Mix	14.4 µl x (N+1)
1.6 µl $MgCl_2$	1.6 µl x (N+1)
2.0 µl Enzyme Mix	2.0 µl x (N+1)

- Place the number of capillaries needed into the respective tray of the real time PCR instrument.
- Pipet **18 µl** of the Master Mix into each capillary.
- Add **2 µl** of the eluates from the DNA isolation (including the eluate of the water control), the Positive Controls and the Negative Control to the corresponding capillary (Table 3).
- Close the capillaries immediately after filling in order to reduce the risk of contamination.

Table 3: Preparation of the real time PCR

Component	Volume
Master Mix	18.0 µl
Sample	2.0 µl
Total Volume	20.0 µl

- Centrifuge capillaries at low speed to collect the prepared reaction volume in the bottom of the capillary.

11.3 Instrument Settings

For the real time PCR and the following analysis of the melting curves use the thermal profile shown in Table 4.

Table 4: real time PCR thermal profile

Description	Time	Temperature	Number of Cycles
Initial Denaturation	10 min	95°C	1
Amplification of DNA			
Denaturation	10 sec	95°C	45
Annealing	10 sec Acquisition mode SINGLE	55°C	
Extension	25 sec	72°C	
	Ramping time for all steps 20°C/sec.		
Melting Curve	0 sec Ramping time 20°C/sec	95°C	
	5 sec Ramping time 20°C/sec	50°C	
	0 sec Ramping time 0.1°C/sec Acquisition mode CONT	80°C	
Cooling	30 sec	40°C	

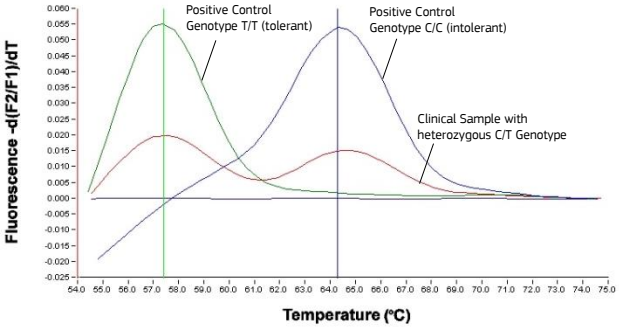
Activate the Color Compensation File (CCC).

12 Data Analysis

For the analysis of the melting curves choose the following adjustments:
Digital filter: enabled
Degrees to average: 9-10

The results of the melting curves analysis for the C/T (-13910) polymorphism are shown in the channel F2 /F1.

Figure 1: Melting curves of homozygous and heterozygous samples.



The melting curves show peaks at a temperature of about 56.5°C for the T/T genotype and about 63.5°C for the C/C genotype. Heterozygous samples (genotype C/T) show peaks at both temperatures.

13 Assay Validation

Negative Controls

All negative controls should be below the threshold. If there is a potential contamination (appearance of a curve in the negative control or a cluster of curves in specimens at high C_T – for example above 36), results obtained are not interpretable and the whole run (including extraction) has to be repeated.

Positive Controls

All the positive controls must show a positive (i.e. exponential) amplification curve. The positive controls must fall below a C_T of 30.
The peaks of the melting curve of the positive controls must located in the temperature range of T/T: 56.5°C +/- 1°C; C/C: 63.5°C +/- 1°C.

14 Limitations of the Method

The results must always be considered in relation to the clinical symptoms. Therapeutical consequences should be made in consideration of clinical data.

15 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@gerbion.com.

No fluorescence signal of the Positive Control	
The selected channel for analysis does not comply with the protocol	Select channel F2/F1 for analysis of the melting curve.
Incorrect configuration of the real time PCR	Check your work steps and compare with ‚Procedure' on page 6.
The programming of the thermal profile is incorrect	Compare the thermal profile with the protocol (Table 4, page 7).
Incorrect storage conditions for one or more kit components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability', page 4.
Weak or no signal in the F2/ F1 channel	
real time PCR conditions do not comply with the protocol	Check the real time RT-PCR conditions (page 7).

real time PCR inhibited	Make sure that you use an appropriate isolation method (see chapter ‚Sample Preparation') and follow the manufacturer's instructions. Make sure that the ethanol-containing washing buffers have been completely removed. An additional centrifugation step at high speed is recommended before elution of the DNA.
DNA loss during isolation process	Make sure that you use an appropriate isolation method (commercial kits are recommended) and stick to the manufacturer's protocol.
Incorrect storage conditions for one or more components or kit expired	Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in ‚Transport, Storage and Stability', page 4.
Detection of a fluorescence signal of the Negative Control	
Contamination during preparation of the real time PCR	Repeat the real time PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Controls at last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that work space and instruments are decontaminated regularly. Use a new kit and repeat the real time PCR.
The peaks of the melting curve of the samples are not located in the temperature range of the control peaks (T/T: 56.5°C +/- 1°C; C/C: 63.5°C +/- 1°C)	
	Existence of a further polymorphism within the region of amplification. The presence of a further polymorphism in the region of amplification may lead to a shift of the melting curves. This can particularly happen in the case of people of non-caucasian origin. Therefore a safe diagnosis cannot be made. If confirmation is necessary, a hydrogen breath test is recommended.

16 Abbreviations and Symbols

DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ENZYME	Enzyme
ENZYME BUFFER	Enzyme Buffer
REACTION MIX	Reaction Mix
CONTROL C/C +	Positive Control Genotype C/C (intolerant)
CONTROL T/T +	Positive Control Genotype T/T (tolerant)
CONTROL −	Negative Control
MGCL2	$MgCl_2$

REF	Catalog number
Σ	Contains sufficient for <n> test
- 18°C	Upper limit of temperature
	Manufacturer
	Use by YYYY-MM
LOT	Batch code
CONT	Content
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
CE	European Conformity

17 Literature

[1] Haberkorn BC et al. Clin Chem Lab Med. 2011;Sept 21: Improving diagnosis of adult-type hypolactasia in patients with abdominal complaints.

[2] Enattah NS et al. Nat Genet. 2001:30(2):223-7: Identification of a variant associated with adult-Type hypolactasia.