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EliGene® Lactose intolerance RT

REF 90092-RT (for 50 samples)

Kit components:

5 x 150 µl **C/T/G/A Mix**
1 x 100 µl **PC DNA C/G**
1 x 100 µl **PC DNA T/A**
Instruction for Use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® Lactose intolerance RT kit is intended for the DNA detection of C-13910T and G-22018A polymorphisms located in the regulatory region of the lactase gene from isolated DNA.

Principle of the method

This diagnostic kit is based on RealTime PCR genotyping (allelic discrimination). In this kit primers and dual-labeled probes (C-13910T FAM and HEX, G-22018A TexasRed and Cy5) for the detection of single nucleotide polymorphisms C-13910T and G-22018A in *MCM6* gene from isolated DNA are used.

Introduction

In clinical laboratories, patients with lactose intolerance are routinely tested for the presence of single nucleotide polymorphisms C-13910T and G-22018A found in the MCM6 gene, which were associated with genetically determined lactose intolerance encompassing decreased lactase production. Genotyping of these polymorphisms allows to differentiate between lactose intolerance caused by genetic predispositions and that caused by secondary effects. Genotyping test facilitates fast and routine analysis of the above-mentioned polymorphisms in a large number of samples. EliGene® Lactose intolerance RT kit provides right results also within patients carrying the C-13907G, T-13913C, G-13914A and T-13915G mutations.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Blood, swabs	Manual: EliGene Urine Isolation Kit (ELISABETH PHARMACON) Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON)

Warning: To keep the sensitivity of the test we recommend to strictly follow the pre-analytical procedures mentioned in this instruction. Specially do not change the recommended procedures as for stated amount of sample, centrifugation force, etc. For the DNA isolation other isolation kits can be used, but the pre-analytical procedures of storage, transport and centrifugation must be held.

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Blood:

Manual isolation:

Add 10 µl of Proteinase K to the sample and then continue according to the standard protocol of EliGene Urine Isolation Kit (ELISABETH PHARMACON) for DNA isolation from blood. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C. For longer period than one week freeze DNA at -20 °C.

Automatic isolation:

Isolate DNA from the sample by using MAGNETO Body fluid DNA/RNA isolation kit according to the protocol for blood samples with elution to 50 µl of Elution buffer.

Swabs:

These samples should be collected according to standard protocol in sterile collection tubes. Samples should be stored and transported at 4 °C. For the diagnostic purposes, it is necessary to isolate DNA from the sample during the day of taking.

Manual isolation:

1. Into 2.0 ml tube pipette 400 µl of MI3 solution and 20 µl of Proteinase K.
2. Put the swab into the 2.0 ml tube and with sterile scissor cut the swab – cut about 0.5 cm above the swab. Close the tube.
3. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently shortly spin the tube.
4. By sterile pincers remove the swab and add 330 µl of solution MI4 to lysate. Vortex and shortly spin.
5. Continue according to the standard protocol of EliGene Urine Isolation Kit. Isolated DNA use immediately for the detection or store it hours to one week at 4 °C. For longer period than one week freeze DNA at -20 °C.

Automatic isolation:

1. Into 2.0 ml tube pipette 450 µl of Lysis buffer, 200 µl of PCR water and 10 µl of Proteinase K.
2. Put the swab into the 2.0 ml tube and with sterile scissor cut the swab – cut about 0.5 cm above the swab. Close the tube.
3. Incubate tube 20 minutes at 56 °C in thermo shaker at 1000 rpm. Consequently, shortly spin the tube.
4. By sterile pincers remove the swab, vortex and shortly spin.
5. Pipette all volume of sample to position “H” in Deep well plate from MAGNETO Body Fluid DNA/RNA isolation Kit.
6. Isolate DNA from the sample by using MAGNETO Body fluid DNA/RNA isolation kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Recommended concentration of analysed DNA is at least 10 ng/µl. It is not recommended to test samples at concentration lower than 10 ng/µl.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for QuantStudio 5 (ThermoFisher Scientific) and CFX96 Touch Real-Time PCR Detection system (Bio-Rad).

- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

Follow the cycler manufacturer's manual when using the kit. Below is a list of cyclers that have been used in testing EliGene® Lactose intolerance RT kit.

- For T allele detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm).
- For C allele detection the probe labeled with HEX is used (exc.520 nm – em. 548 nm).
- For G allele detection the probe labeled with TxRed is used (exc. 589 nm – em. 615 nm).
- For A allele detection the probe labeled with Cy5 is used (exc. 650 nm – em. 670 nm).

QuantStudio 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type," Genotyping", Chemistry "TaqMan Probes", and Run Mode "Standard".

Set up the following temperature profile:

Holding stage

95°C 3 min Ramp rate (1.6°C/s)

Cycling stage – 39 cycles

95°C 10 s Ramp rate (1.6°C/s)

58°C 10 s Ramp rate (1.6°C/s) Data collection ON

67°C 20 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 1 min Ramp rate (1.6°C/s)

Collect emission signal at the second step of cycling stage at 58 °C.

According to the cycler manufacturer's instructions, enter the individual alleles with the appropriate reporter colors for the plate - allele 13910 T (FAM), C (VIC); allele 22018 G (ROX), A (Cy5) and select NONE as the passive reference dye.

The complete temperature profile can be up-loaded from Run Template "EliGene_LAC_QS5_v00.edt", which you can download on the website www.elisabeth.cz in the Documents section.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1	95°C	3 min
Step 2	95°C	10 s
Step 3	58°C	10 s + Plate Read
Step 4	67°C	20 s
Step 5	*GOTO Step 2*	*39x*
Step 6	40°C	60 s

Enter the Sample Volume 20 µl.

Collect emission signal at the Step 3 at 58° C.

For filter settings use the "Scan Mode" All Channels but in Plate Manager select for the samples only fluorophores

FAM, HEX, TxRed, Cy5. Then assign the samples with positions and Targets FAM, HEX, TxRed and Cy5 as an Unknown sample (Samples) or Standard. The complete temperature profile can be up-loaded from Run Template "EliGene_LAC_CFX_v00.prcl", which you can download on the website www.elisabeth.cz in the Documents section.

If using instruments other than the validated ones listed in this manual, the parameters for reading and analyzing the results must be set for such instrument. These settings must be verified by comparison with the results obtained on one of the validated instruments. For comparison purposes, reference samples or samples from external quality assessment can be used. If the parameters for reading and analysis of the results are not verified, the criteria for interpretation of the results given in the manual cannot be applied to non-validated instruments, as incorrect results may be given. In these cases, the manufacturer does not bear responsibility for any issues related to incorrect result interpretation.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mixed on vortex and shortly spun.
- **If you do not use all the volume of MasterMix, store the tube at dark at temperature 4°C up to 14 days. For long-term storage use the freezer (-20 °C, dark). MasterMix should not go through more than five freeze- thaw cycles.**

Preparation of Reaction Mix for Quant Studio 5 and CFX96

1. Detection: Take one microtube with C/T/G/A Mix and after the thawing, vortexing and quick spin pipette 15 µl of the mix to amplification microtube or plate and add 5 µl of isolated DNA.
2. Standards: Take one microtube with C/T/G/A Mix and after the thawing pipette 15 µl of mix to amplification microtube or plate and 5 µl of individual standards.

Insert the micro tubes or plate to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Result reading

QuantStudio 5 Real-Time PCR Systems (ThermoFisher Scientific):

In "Analysis Settings" choose for both alleles in folder "Call Settings" the option "Analyze Real-Time dRn data" and in folder "Ct Settings" select "Baseline Start Cycle 3 – end cycle 15" option and analyze results by selecting "**Apply**".

The software will automatically evaluate the presence of individual alleles.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose "Allelic Discrimination". In "Settings" menu choose the option "Baseline Threshold" and choose in "Baseline Cycles" the option "Auto Calculated" and in "Single Threshold" the option "Auto Calculated".

In the window Allelic Discrimination choose the appropriate combination of fluorophores for a given allele. 13910 – FAM (T), HEX (C), 22018 - TxRed (G), Cy5 (A). The software will automatically evaluate the genotype based on the RFU values.

Samples with RFU values <500 for both fluorophores are not evaluable.

Set the fluorophores as follows:

X= FAM
Y= HEX

Allele 1=genotype TT, Allele 2 = genotype CC

X= TxRed
Y= Cy5

Allele 1 = genotype GG, Allele 2 = genotype AA

Interpretation of results

Allele	***Lactose intolerance (decreased lactase activity)***	***Lactose tolerance***	
13910T	CC	TT	CT
22018A	GG	AA	GA

Control procedure

EliGene® Lactose intolerance RT Kit involves Positive Controls (PC DNA C/G, PC DNA T/A).

Positive control follows the proper function of MasterMix. Minimal Cp of positive control must be 30 or less. The Cp higher than 30 for positive control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Reference material:

To monitor all examination process covering DNA isolation and RealTime PCR detection is possible to use reference material positive for individual genotypes. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification in any of the channels, there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

EliGene® Lactose intolerance RT kit specifically detects the CC, CT and TT genotype of C-13910T polymorphism and the GG, GA and AA genotype of G-22018A polymorphism. The kit detects human genomic DNA of concentration higher than 10 ng/µl.

Analytical sensitivity is 10 ng of DNA in reaction mix.

Analytical specificity of method is 100%. Analytical specificity of the method was validated by searching in the DNA databases.

Clinical specificity EliGene® Lactose intolerance RT kit specificity was tested on 31 samples of human DNA with genotypes determined by reference method.

Diagnostic performance characteristics:

EliGene® Lactose intolerance RT kit specificity was tested using 31 samples of human DNA analyzed by reference method, when 8 samples were positive for the risk genotype. 100 % agreement was achieved when comparing the test results.

The analytical specificity of EliGene® Lactose intolerance RT kit is 100%.

Measuring interval

The kit enables the detection of ≥ 10 ng DNA molecules in reaction mix.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

Unused content of the tube with MasterMix is stable at -20 °C. Do not freeze tubes with MasterMix more than 5 times! Do not mix components of the kits of different lots.

Warnings and general precautions

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.

- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box (not laminar flow box). The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination.

Warnings and precautions specific to components of the kit

- The tubes containing mixes are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing positive controls are disposable and therefore must be used once only.
- These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

In the case of any problems, contact the customer center of ELISABEHT PHARMACON, spol. s r.o.

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Literature

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Symbols

	Catalog number
	Upper limit of temperature
	Batch code
	Use by (last day of month)
	*in vitro* diagnostic medical device
	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Consult instruction for use
	Manufacturer

Manufacturer

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