ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

EliGene® Coeliac RT (DQ2, DQ8, DR4)

REF 90048-RT **(for 50 samples)**

Kit components:

5 x 180 µl **CELI-DQ2 Mix**
5 x 180 µl **CELI-DQ8 Mix**
5 x 180 µl **CELI-DR4 Mix**
2 x 50 µl **PC CELI**
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® Coeliac RT is intended for the genotypization of HLA-DQ2, HLA-DQ8 and HLA-DR4 genes from isolated DNA.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and labeled probes (FAM and JOE) for the detection of DQ2, DQ8 and DR4 alleles and for the detection of internal control are used.

Introduction

Celiac disease, also called celiac sprue is one of the most common enteropathogenic disorders and is characterized by a lifelong hypersensitivity to gluten proteins found in wheat, rye, oat and barley. Celiac disease does not belong to allergic disease but it is characterized by intolerance to gliadin forming parts of gluten. In early childhood the immunological intolerance to gliadin, leads to a chronic inflammatory response in the small-intestinal mucosa and subsequent malabsorption characterized by chronic diarrhea, steatorrhea and failure to thrive. Many adult patients can also show other atypical signs such as, abdominal distention, weight loss, fatigue, skin- and joint problems or migraine-like headache. Indeed, others may remain largely asymptomatic. Non-treated celiac disease increases the risk of NHL (non-Hodgkin's lymphoma) and probably small-intestinal cancer.

Until recently, celiac disease was considered relatively uncommon with an estimated prevalence rate ranging from 1 in 1000 to 1:4000. However, the availability of new serologic tests have led to the observation that celiac disease is much more common, affecting about 1 of 100-400 persons in Europe, the majority of patients showing little clinical symptoms. Considerable evidence now indicates that celiac disease has a strong genetic component.

Celiac disease is a multifactorial disorder associated with HLA-DQ2 (DQA1*05/DQB1*02) or DQ8 (DQA1*0301/DQB1*0302) that is associated in haplotype with HLA-DR4 allele. HLA DQ2 is expressed in the majority (> 90 %) of those with celiac disease and DQ8 in about 8%**. The expression of these HLA-DQ2 or DQ8 molecules is necessary but not sufficient to develop celiac disease and accounts for only about 50% of the genetic component of the disease**. Studies in sibling (sib recurrence risk for celiac disease of 10%) and of identical twins (concordance of 70%) suggest that the contribution of HLA genes in celiac disease is less than 50%. About 95% of all celiac patients possess this particular genotype compared to approximately 20% of the normal population. Of the few celiac patients who are negative for HLA-DQ2 (DQA1*05/DQB1*02), a great majority are HLA-DQ8 positive**. From this point of view absence of these alleles is useful in excluding celiac disease**.

However, at least one other non-HLA gene and some environmental factors are also likely to be involved in the disease.

EliGene® Coeliac RT kit detects alleles HLA-DQ2 (DQA1*05, DQB1*02), HLA-DQ8 (DQA1*03, DQB1*0302) and HLA-DR4 (DRB1*04). As an internal control the gene SYPL2 (synaptophysin-like 2) is used.

Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Blood	Manual: EliGene Urine Isolation Kit (ELISABETH PHARMACON) Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON) Prepito NA Body Fluid Kit (Chemagen-PerkinElmer)
Swabs	Manual: EliGene Urine Isolation Kit (ELISABETH PHARMACON) Automatic: **ZEPHYRUS Magneto** (ELISABETH PHARMACON) Prepito NA Body Fluid Kit (Chemagen-PerkinElmer)

Blood (collection into tube with EDTA):

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 or freeze DNA at -20 °C for longer period than one week.

Automatic isolation:

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

Buccal swabs:

These specimens should be collected according to standard protocol in collection tubes. Specimens should be transported to the laboratory 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 or freeze DNA at -20 °C for longer period than one week.

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 at Deep well plate from Magneto Body Fluid Kit.
6. Isolate DNA from the sample by using Magneto Body fluid kit according to protocol for plasma samples with elution to 50 µl of Elution buffer.

Recommended concentration of analysed DNA is 10–50 ng/µl. It is not recommended to test samples at concentration lower than 5 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 LightCycler® 480 (Roche), QuantStudio 3 a 5 Real-Time PCR system (ThermoFisher Scientific), ABI7500FAST (Applied Biosystems), Rotor-Gene Q (Qiagen) a CFX96 Touch Real-Time PCR Detection system (Bio-Rad). The qPCR utilizes TaqMan technology (FAM and JOE labelled probes) and can be performed on other instruments that can work in FAM and HEX channels.
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For DQA1*05, DRB1*04 and DQB1*0302 alleles detection the probe labeled with FAM is used (abs. 494 nm – emission 518 nm).
- For DQA1*03, DQB1*02 and SYPL2 (internal control) alleles detection the probe labeled with JOE is used (similar to VIC – abs. 525 nm – emission 548 nm).
- Reaction Mix includes passive reference control dye High-ROX for signal normalization.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose “Dual Color Hydrolysis probe”.

Set up the following temperature profile:

Step 1 - Analysis mode “None”, 1 Cycle

95°C	3 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode “Quantification”, 40 cycles

95°C	15 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
60°C	40 s	Ramp rate (2.2°C/s)	Acquisition mode “Single”

Step 3 - Analysis mode “None”, 1 Cycle

40°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode “None”

The complete temperature profile can be up-loaded from Run Template “CELI_RT_LC480_v03.ixo”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type," Presence/Absence", 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 – 40 cycles

95°C 15 s Ramp rate (1.6°C/s)

60°C 40 s Ramp rate (1.6°C/s) Data collection ON

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect emission signal at the second step at 60 °C

The complete temperature profile can be up-loaded from Run Template "EliGene_CELI_RT_QS3_v00.edt" nebo "EliGene_CELI_RT_QS5_v00.edt". The Run Template can be copied from the CD included in the kit.

RealTime System ABI7500FAST (Applied Biosystems):

Use the program module for absolute quantification ("Quantitation-Standard Curve") and "7500 (96wels)" instrument type.

Set up the following temperature profile:

Holding stage

95°C 3 min

Cycling stage – 40 cycles

95°C 15 s

60°C 40 s Data collection ON

Holding stage

40°C 20s

Collect emission signal at the second step – 60 °C.

RotorGene Q (Qiagen):

In the "New Run" window choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

95°C 3 min

Cycling stage – 40 cycles

95°C 15 s

60°C 40 s Acquiring in channels "Green" and "Yellow"

Holding stage

40°C 20 s

For the Gain optimization in all channels select option "Automatic gain optimization before first acquisition". The

complete temperature profile can be up-loaded from Run Template "EliGene_CELI_RT_Q-GENE_v01.ret". The Run Template can be copied from the CD included in the kit.

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	15 s
Step 3	60°C	40 s + Plate Read
Step 4	*GOTO Step 2*	*40x*
Step 5	40°C	20 s

Enter the Sample Volume 20ul

Collect emission signal at the Step 3 at 60° C.

For filter settings use the "Scan Mode" All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard. The complete temperature profile can be up-loaded from Run Template "EliGene_CELI_RT_CFX_v00.prcl". The Run Template can be copied from the CD included in the kit.

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 mix on vortex and shortly spin.
- Isolate DNA according to standard protocol.

Preparation of Reaction Mix

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

1. Detection: Take one microtube with CELI-DQ2 Mix, one microtube with CELI-DQ8 Mix and one microtube with CELI-DR4 Mix and after the thawing pipette 17.5 µl of each Mix to separate amplification microtube. Thereafter add 2.5 µl of isolated DNA to each microtube. During the pipetting of samples be careful to avoid cross-contamination of samples. If you do not use all the volume of MasterMix, freeze it and store at -20 °C in a dark. Do not freeze tubes with MasterMix repeatedly. Under these conditions it is stable at least for 14 days.
2. Positive control: Take one microtube with CELI-DQ2 Mix, one microtube with CELI-DQ8 Mix and one microtube with CELI-DR4 Mix and after the thawing pipette 17.5 µl of each Mix to amplification microtube and add 2.5 µl of Positive Control DNA (PC CELI).

Insert the microtubes or plate with samples to the RealTime PCR instrument and run the program according to chapter “Configuration of Real Time instrument” above.

Result reading

LightCycler® 480 (Roche):

In menu “Analysis” choose “Abs Quant/2nd Derivative Max” option.

In Analysis window click to “Color Comp” icon and choose Universal CC FAM (510)-HEX (580) calibration. Analyze results by clicking to icon “Calculate”.

Positive result: The positive results are characterized by amplification and growths of signal in FAM channel (465-510) and in JOE channel (533-580). In a case of negative results the amplification will not occur.

The values of Concentration correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

QuantStudio 3 and 5 Real-Time PCR Systems (ThermoFisher Scientific):

In “Analyse Settings” choose for “Baseline“ Start Cycle 3 and End Cycle 15. The value of “Threshold“ set-up to 1/3rd of maximal intensity of positive control signal for given allele.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm) and in JOE channel (em. 548 nm). In a case of negative results, the amplification will not occur.

The values of Qty correspond to the quantity of positive result, “result undet.” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

RealTime Systems ABI 7500FAST (Applied Biosystems):

In “Analyse Settings” choose for “Baseline“ Start Cycle 3 and End Cycle 15. The value of “Threshold“ set-up to 1/3rd of maximal intensity of positive control signal for given allele.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm) and in JOE channel (em. 548 nm). In a case of negative results, the amplification will not occur.

The values of Qty correspond to the quantity of positive result, “result undet.” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Rotor-Gene Q (Qiagen):

Click to “Analysis” icon in the menu and choose Analysis option “Quantitation”. In “Quantitation Analysis” window choose “Dynamic Tube” and “Slope Correct” option. In menu “Outlier Removal” set-up NTC Threshold to value of 10 %. The value of “Threshold“ set-up to 1/3rd of maximal intensity of positive control signal for given allele.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel (Green) and in JOE channel (Yellow). In a case of negative results, the amplification will not occur.

The values of “Calc. conc.” correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

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

In Data Analysis window choose “Quantification”. In “Settings” menu choose option “Baseline Threshold” and select “Baseline Cycles” option as “Auto Calculated” and “Single Threshold” option as “User defined” to the 1/3rd of maximal intensity of positive control signal for given allele.

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result: The positive results are characterized by amplification and growth of signal in FAM channel and in HEX channel. In a case of negative results, the amplification will not occur. The values of Concentration correspond to the quantity of positive result; "Negative" means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

Interpretation of results

*HLA-DQ2 (DQA1*05/DQB1*02) positive*

If the increasing of amplification signal in CELI-DQ2 Mix appears in both channels, FAM (DQA1*05) and JOE (DQB1*02), before the cycle number 35.

*HLA-DQ2 (DQA1*05/DQB1*02) negative*

If the increasing of amplification signal in CELI-DQ2 Mix does not appear in both channels, FAM (DQA1*05) and JOE (DQB1*02), before the cycle number 35. The signal in CELI-DR4 Mix in JOE channel for the internal control (SYPL2) must be positive.

*Important notice: For HLA-DQ2 positivity it is necessary to have positivity for both alleles, DQA1*05 and DQB1*02. If only one allele is present the patient is not HLA-DQ2 positive.*

*HLA-DQ8 (DQA1*03/DQB1*0302) positive*

If the increasing of amplification signal in CELI-DQ8 Mix appears in both channels, FAM (DQB1*0302) and JOE (DQA1*03), before the cycle number 35.

*HLA-DQ8 (DQA1*03/DQB1*0302) negative*

If the increasing of amplification signal in CELIDQ8 Mix does not appear in both channels, FAM (DQB1*0302) and JOE (DQA1*03), before the cycle number 35. The signal in CELI-DR4 Mix in JOE channel for the internal control (SYPL2) must be positive.

*Important notice: For HLA-DQ8 positivity it is necessary to have positivity for both alleles, DQA1*03 and DQB1*0302. If only one allele is present the patient is not HLA-DQ8 positive.*

HLA-DR4 positive

If the increasing of amplification signal in CELI-DR4 Mix appears in channel FAM (DRB1*04) before the cycle number 35 the sample is HLA-DRB1*04 positive.

*Important notice: Due to the fact that DR4 allele is present in haplotype with DQ8 allele, in case of detected DQ8 allele the DR4 allele is detected as well. Only very rarely is the DQ8 allele positive without DRB1*04.*

HLA-DR4 negative

If the increasing of amplification signal in CELI-DR4 Mix does not appear in channel FAM before cycle number 35, the sample is HLA-DRB1*04 negative. The signal in CELI-DR4 Mix in JOE channel for the internal control (SYPL2) must be positive.

Positive result:

If at least one allele (HLA DQ2 and/or HLA DQ8) was detected in the sample, the cealiac disease may occur and develop.

The genetic component is estimated to be approximately 50%

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Negative result

If the sample is not positive for HLA DQ2 or HLA DQ8, the celiac disease can be ruled out.

If the sample is positive only for HLA-DRB1*04, the celiac disease can be ruled out.

Inhibited sample:

If in the JOE channel of CELI-DR4 Mix there is no increase of amplification signal. In this case it is necessary to repeat the analysis. The best way is to use DNA samples prepared by new DNA isolation.

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

The following table summarizes the possible results.

Genotyp	CELI-DQ2 Mix			CELI-DQ8 Mix			CELI-DR4 Mix	
	FAM DQA1*05	JOE DQB1*02	FAM DQB1*0302	JOE DQA1*03	FAM DRB1*04	JOE IC
DQ2	+	+	-	+	-	+
DQ2	+	+	-	+	+	+
DQ2	+	+	+	-	+	+
DQ2	+	+	+	-	-	+
DQ8/DR4	+	-	+	+	+	+
DQ8/DR4	-	+	+	+	+	+
DQ8/DR4	-	-	+	+	+	+
DQ2/DQ8/DR4	+	+	+	+	+	+
DR4	+	-	-	-	+	+
DR4	-	+	-	-	+	+
DR4	-	-	-	+	+	+
DR4	+	-	-	+	+	+
DR4	-	+	-	+	+	+
DR4	-	-	-	+	+	+

Control procedure

EliGene® Coeliac RT kit as an internal isolation control detects the human gene SYPL2 (synaptophysin-like 2) in CELI-DR4 Mix in JOE channel. SYPL2 gene is present in each human DNA sample, so it is not necessary to add internal control to the sample. Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is DQA1*05/DQB1*02, DQA1*03/DQB1*0302 and DRB1*04 negative, the Ct of internal control must be Ct < 35.

Reference material:

To monitor all examination process covering DNA isolation and RealTime PCR detection is possible to use reference material positive for DQ2, DQ8 and DR4 genes. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control, 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® Coeliac RT kit is specific for detection of DQA1*05, DQA1*03, DQB1*02, DQB1*0302 and DRB1*04 alleles. The kit detects human genomic DNA of concentration higher than 5 ng/µl added to the amplification mix.

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® Coeliac RT kit specificity was tested on 50 samples of human DNA with genotypes determined by DNA sequencing.

Diagnostic performance characteristics:

EliGene® Coeliac RT kit specificity was tested on 50 samples of human DNA with genotypes determined by DNA sequencing. Totally 50 samples were right determined by EliGene® Coeliac RT kit.

The clinical specificity of EliGene® Coeliac RT kit is 100%.

Measuring interval

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

Internal control of quality

As an internal control of quality the Internal Control for checking the process of DNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

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 for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! 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 treated with 3% sodium hypochlorite for at least 30 minutes or 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 or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. 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. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mixes (CELI-DQ2 Mix, CELI-DQ8 Mix and CELI-DR4 Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- 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.

Literature

Bourgey M, Calcagno G, Tinto N, Gennarelli D, Margaritte-Jeannin P, Greco L, Limongelli MG, Esposito O, Marano C, Troncone R, Spampanato A, Clerget-Darpoux F, Sacchetti L. (2007) HLA related genetic risk for coeliac disease. *Gut*. 56(8):1054-9

Margaritte-Jeannin P, Babron MC, Bourgey M, Louka AS, Clot F, Percopo S, Coto I, Hugot JP, Ascher H, Sollid LM, Greco L, Clerget-Darpoux F. (2004) HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease. *Tissue Antigens* 63(6):562-7.

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
	Attention, consult instructions for use
	Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827
E-mail: info@elisabeth.cz