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The company is registered in the Commercial Register at the Regional Court in Brno under file number C 40647.

EliGene® CELIAC DQ PLUS RT

REF **90085-RT (for 50 samples)**

Kit components:

5 x 150 µl **HLA-DQ2 Mix**
5 x 150 µl **HLA-DQ8/DR4 Mix**
2 x 50 µl **PC CELI**
Instructions for Use

Storage and shelf life:

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® CELIAC DQ PLUS RT is intended for the genotyping of HLA-DQ2.5 (DQA1*05/DQB1*02), HLA-DQ2.2 (DQA1*02/DQB1*02), HLA-DQ8 (DQA1*03/DQB1*03:02) and HLA-DRB1*04 alleles from isolated DNA.

Principle of the method

This diagnostic kit is based on the RealTime PCR method. In this kit primers and labelled probes (FAM, HEX, TexasRed and Cy5) for the detection of DQ2.5, DQ2.2, DQ8 and DRB1*04 alleles and 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 diarrhoea, 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 few clinical symptoms. Considerable evidence now indicates that celiac disease has a strong genetic component.

Celiac disease is a multifactorial disorder associated with HLA-DQ2.5 (DQA1*05/DQB1*02) or DQ8 (DQA1*03/DQB1*03:02) that is associated in haplotype with HLA-DR4 allele. HLA DQ2.5 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-DQ2.5 and HLA-DQ8, a great majority are HLA-DQ2.2 (DQA1*02/DQB1*02) 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® CELIAC DQ PLUS RT detects alleles HLA-DQ2.5 (DQA1*05/DQB1*02), HLA-DQ2.2 (DQA1*02/DQB1*02), HLA-DQ8 (DQA1*03/DQB1*03:02) and HLA-DRB1*04. As an internal control, the one-copy 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)
Swabs	Manual: EliGene Urine Isolation Kit (ELISABETH PHARMACON) Automatic: ZEPHYRUS Magneto (ELISABETH PHARMACON)

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 uses immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for a longer period than one week.

Automatic isolation:

Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kit according to the 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 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 the lysate. Vortex and shortly spin.
5. Continue according to the standard protocol of EliGene Urine Isolation Kit. Isolated DNA uses immediately for the detection or store it hours to one week at 4 °C or freeze DNA at -20 °C for a 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 BodyFluid DNA/RNA isolation kit.
6. Isolate DNA from the sample by using MAGNETO BodyFluid DNA/RNA isolation kitkit according to the protocol for plasma samples with elution to 50 µl of Elution buffer.

The recommended concentration of analyzed DNA is 1–10 ng/µl. It is not recommended to test samples at a lower concentration of than 1 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 qPCR – the kit is designed for qPCR instruments QuantStudio 5 (ThermoFisher Scientific), Rotor-Gene Q (Qiagen), CFX96 (Bio-Rad) and the MIC cycler (Bio Molecular Systems).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real-Time instrument

- For detection of alleles DQA1*05 and DQB1*03:02 the probe labelled with FAM (exc. 494 nm – em. 518 nm) is used.
- For detection of alleles DQB1*02 and DQA1*03 the probe labelled with HEX (exc. 520 nm – em. 548 nm) is used.
- For detection of alleles DQA1*02 and DRB1*04 the probe labelled TexasRed (exc. 589 nm – em. 615 nm) is used.
- For detection of the SYPL2 gene (internal control), the probe labelled with Cy5 (exc. 650 nm – em. 670 nm) is used.

QuantStudio 5 (ThermoFisher Scientific):

Use the Experiment type," **Presence/Absence**", Chemistry "**TaqMan Probes**", and Run Mode "**Standard**". As a reporter dyes use **FAM** (DQA1*05 and DQB1*03:02), **VIC** (DQB1*02 and DQA1*03), **ROX** (DQA1*02 and DRB1*04) and **Cy5** (SYPL2 gene). **DO NOT USE ANY PASSIVE REFERENCE DYE!**

Set up the following temperature profile:

Holding stage

95°C 3 min Ramp rate (1.6°C/s)

Cycling stage – 40 cycles

95°C 10 s Ramp rate (1.6°C/s)

59°C 20 s Ramp rate (1.6°C/s) Data collection ON

67°C 30 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect the emission signal at the second step at 59 °C

The complete temperature profile can be uploaded from Run Template "EliGene CELIAC DQ PLUS RT_QS5.edt". The Run Template can be copied from the CD included in the kit.

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 10 s

59°C 20 s Acquiring in channels "Green", "Yellow", "Orange" and "Red"

67°C 30 s

Holding stage

40°C 20 s

For the Gain optimization in all channels, select the option "Automatic gain optimization before first acquisition". The complete temperature profile can be uploaded from Run Template "EliGene CELIAC DQ PLUS RT_Q-GENE.ret". The Run Template can be copied from the CD included in the kit.

CFX96 Touch (Bio-Rad):

In Startup Wizard Create a new Experiment for the 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	59°C	20 s	+ Plate Read
Step 4	67°C	30 s	
Step 5	*GOTO Step 2*	*39x*	
Step 6	40°C	20 s	

Enter the Sample Volume of 20 µl

Collect the emission signal at Step 3 at 59° C.

For filter settings, use the "Scan Mode" All Channels, and in Plate Manager, select for the samples only fluorophores FAM, HEX, TexasRed, and Cy5. Then assign the samples with positions and Targets as an Unknown sample (Samples) or Standard (Positive control). The complete temperature profile can be uploaded from Run Template "EliGene CELIAC DQ PLUS RT_CFX96.prcd". The Run Template can be copied from the CD included in the kit.

MIC Cycler (Bio Molecular Systems):

In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 20µl and Temperature Control "Fast TAQ (v3)".

Set up the following temperature profile:

Hold

95°C 3 min

Cycling – 40 cycles

95°C 10 s

59°C 10 s Acquire on "Green", "Yellow", "Orange" and "Red"

67°C 20 s

Hold after cycling

40°C 20 s

Acquire data at the end of step 59°C for "Green", "Yellow", "Orange" and "Red" channel.

The complete temperature profile can be uploaded from Run Template "EliGene CELIAC DQ PLUS RT_MIC.mictemplate". 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 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: CONTAMINATION IN LABORATORY SPACE IS POSSIBLE. USE SEPARATE PIPETTE FOR MASTER MIXES, SEPARATE PIPETTE FOR POSITIVE CONTROLS, AND SEPARATE PIPETTE FOR SAMPLES! FOLLOW ALL RECOMMENDATIONS FOR LABORATORIES PROVIDING RNA ANALYSES.

1. Detection: Take one microtube with HLA-DQ2 Mix and one microtube with HLA-DQ8/DR4 Mix and after the thawing pipette 15.0 µl of each Mix to separate amplification microtube. Thereafter add 5.0 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 it at -20 °C in a dark. **Freeze/thaw tubes with MasterMix for max. 5-times**.
2. Positive control: Take one microtube with HLA-DQ2 Mix and one microtube with HLA-DQ8/DR4 Mix and after the thawing pipette 15.0 µl of each Mix to amplification microtube and add 5.0 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

QuantStudio 5 (ThermoFisher Scientific):

For the HLA-DQ2 mix in “**Analysis Settings**” edit default Ct settings for each channel (FAM, VIC, ROX, Cy5) and enter threshold value corresponding to **20% of the maximal signal intensity of positive control** for selected channel and “Automatic Baseline” option. After finishing of manual Ct setting for each channel click on “**Apply**” to accept the current settings and analyze results.

Positive result: The positive result is characterized by amplification and growth of signal in FAM (exc. 494 nm – em. 518 nm), HEX (exc. 520 nm – em. 548 nm), TexasRed (exc. 589 nm – em. 615 nm) or Cy5 (exc. 650 nm – em. 670 nm) channel. In the case of a negative result, the amplification will not occur.

For the HLA-DQ8/DR4 mix in “**Analysis Settings**” edit default Ct settings for each channel (FAM, VIC, ROX, Cy5) and preserve **“Default settings”** for all the channels. After that click on “**Apply**” to accept the current settings and analyse results.

Positive result: The positive result is characterized by amplification and growth of signal in FAM (exc. 494 nm – em. 518 nm), HEX (exc. 520 nm – em. 548 nm), TexasRed (exc. 589 nm – em. 615 nm) or Cy5 (exc. 650 nm – em. 670 nm) channel. In the case of a negative result, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control (SYPL2 gene) amplification is characterized by the growth of the fluorescence signal in the Cy5 channel.

Rotor-Gene Q (Qiagen):

Click to "Analysis" icon in the menu and choose the Analysis option "Quantitation". In the "Quantitation Analysis" window, select the "**Dynamic Tube**" and "**Slope Correct**" option. In the option "**Outlier Removal**" setup **NTC Threshold value for 20 %**.

Positive result: The positive result is characterized by amplification and growth of signal in FAM (Green), HEX (Yellow), TexasRed (Orange) or Cy5 (Red) channel. In the case of a negative result, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control (SYPL2 gene) amplification is characterized by the growth of the fluorescence signal in the Cy5 (Red) channel.

CFX96 Touch (Bio-Rad):

In the Data Analysis window, choose "Quantification". In the "Settings" menu, choose the "**Baseline Setting**" option, and select "**Baseline Subtracted Curve Fit**" and "**Apply Fluorescence Drift Correction**".

For the HLA-DQ2 mix in "**Settings**" select "**Baseline Threshold**" and set the "**Single Threshold**" baseline to the value corresponding to **20% of the maximal signal intensity of positive control** for the selected channel. The positive result is characterized by the growth of the fluorescence signal in the FAM, HEX, TexasRed or Cy5 channel. In the case of negative results, the amplification will not occur.

For the HLA-DQ8/DR4 mix in "**Settings**" select "**Baseline Threshold**" and set the "**Single Threshold**" baseline to **“Auto Calculated”** for the selected channel. The positive result is characterized by the growth of the fluorescence signal in the FAM, HEX, TexasRed or Cy5 channel. In the case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control (SYPL2 gene) amplification is

characterized by the growth of the fluorescence signal in the Cy5 channel.

MIC Cycler (Bio Molecular Systems):

Under Analysis, click the small arrow next to Cycling and then select the target - FAM (Green), HEX (Yellow), TexasRed (Orange) or Cy5 (Red). In the down right corner **increase** "**Fluorescence Cut-off Level**" **to 20 %**. A quantification cycle (Cq) value is displayed for each positive well. This is the cycle number at which the sample fluorescence rises above the threshold (the red horizontal line near the bottom of the amplification curve chart). In the Samples pane on the right, click sample names to hide or display the associated amplification curves and result data.

Positive result: The positive result is characterized by amplification and growth of signal in FAM (Green), HEX (Yellow), TexasRed (Orange) or Cy5 (Red) channel. In the case of a negative result, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control (SYPL2 gene) amplification is characterized by the growth of the fluorescence signal in the Cy5 (Red) channel.

Interpretation of results

*HLA-DQ2.5 (DQA1*05/DQB1*02) positive*

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

*HLA-DQ2.5 (DQA1*05/DQB1*02) negative*

If the increasing of amplification signal in HLA-DQ2 Mix does not appear in both channels FAM (DQA1*05) and HEX (DQB1*02) before cycle number 35. In the Cy5 channel the signal for the internal control (SYPL2) must be positive.

*IMPORTANT NOTICE: For HLA-DQ2 positivity is necessary to have both alleles DQA1*05 and DQB1*02. If only one allele is present, the patient is not HLA-DQ2.5 positive.*

*HLA- DQ2.2 (DQA1*02/DQB1*02) positive*

If the increasing of amplification signal in HLA-DQ2 Mix appears in both channels HEX (DQB1*02) and TexasRed (DQA1*02) before cycle number 35.

*HLA- DQ2.2 (DQA1*02/DQB1*02) negative*

If the increasing of amplification signal in HLA-DQ2 Mix does not appear in both HEX (DQB1*02) and TexasRed (DQA1*02) before cycle number 35. In the Cy5 channel,the signal for the internal control (SYPL2) must be positive.

*IMPORTANT NOTICE: For HLA-DQ2.2 positivity it is necessary to have positivity for both alleles, DQA1*02 and DQB1*02. If only one allele is present, the patient is not HLA-DQ2.2 positive.*

*HLA-DQ8 (DQA1*03/DQB1*03:02) positive*

If the increasing of amplification signal in HLA-DQ8/DR4 Mix appears in both channels, FAM (DQB1*03:02) and HEX (DQA1*03), before the cycle number 35.

*HLA-DQ8 (DQA1*03/DQB1*03:02) negative*

If the increasing of amplification signal in HLA-DQ8/DR4 Mix does not appear in both channels, FAM (DQB1*03:02) and HEX (DQA1*03), before the cycle number 35. The signal for 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*03:02. If only one allele is present, the patient is not HLA-DQ8 positive.*

*HLA-DRB1*04 positive*

If the increasing of amplification signal in HLA-DQ8/DR4 Mix appears in channel TexasRed (DRB1*04) before cycle number 35, the sample is HLA-DRB1*04 positive.

*IMPORTANT NOTICE: Since the DR4 allele is found in the same haplotype as the DQ8 allele, the detection of DQ8 allele positivity also results in the detection of DR4 allele positivity. Only very rarely is the DQ8 allele positive without DRB1*04.*

*HLA-DRB1*04 negative*

If the increasing of amplification signal in HLA-DQ8/DR4 Mix does not appear in channel TexasRed before cycle number 35, the sample is HLA-DRB1*04 negative. The signal for internal control (SYPL2) must be positive.

Positive result:

If at least one allele (HLA DQ2.5 and/or DQ2.2 and/or HLA DQ8) was detected in the sample, the disease may occur and develop. The genetic component is estimated to be approximately 50%

WARNING: 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 DQ2.5 and/or HLA DQ2.2 and/or HLA DQ8, the celiac disease can be ruled out. If the sample is positive only for HLA-DRB1*04, celiac disease can be excluded.

Inhibited sample:

If in the Cy5 channel of HLA-DQ2 and HLA-DQ8/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.

Control procedure

EliGene® CELIAC DQ PLUS RT kit as an internal isolation control detects the human gene SYPL2 (synaptophysin-like 2) in Cy5 channel of both mixes, HLA-DQ2 and HLA-D Q8/DR4. SYPL2 gene is present in one copy 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 inhibition of the amplification process. In the case that the sample is DQA1*05, DQB1*03:02, DQB1*02, DQA1*03, DQA1*02 and DRB1*04, 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 DQ2.5, DQ2.2, DQ8 and DRB1*04 alleles. 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 a RealTime instrument breakdown.

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The company is registered in the Commercial Register at the Regional Court in Brno under file number C 40647.

The following table summarizes the results showing a genetic predisposition for coeliac disease.

	HLA-DQ2 Mix				HLA-DQ8/DR4 Mix			
Genotype	FAM (DQA1*05)	HEX (DQB1*02)	TxR (DQA1*02)	Cy5 (IC)	FAM (DQB1*03:02)	HEX (DQA1*03)	TxR (DRB1*04)	Cy5 (IC)
DQ2.5	+	+	-	+	-	+	-	+
DQ2.5	+	+	-	+	-	-	-	+
DQ2.5/DR4	+	+	-	+	-	+	+	+
DQ2.5/DR4	+	+	-	+	-	-	+	+
DQ2.2	-	+	+	+	-	+	-	+
DQ2.2	-	+	+	+	+	-	-	+
DQ2.2/DR4	-	+	+	+	-	+	+	+
DQ2.2/DR4	-	+	+	+	-	-	+	+
DQ8	-	+	-	+	+	+	+	+
DQ8	+	-	+	+	+	+	+	+
DQ8	-	-	+	+	+	+	+	+
DQ8	-	-	-	+	+	+	+	+
DQ2.5/DQ2.2	+	+	+	+	-	+	-	+
DQ2.5/DQ2.2	+	+	+	+	-	-	-	+
DQ2.5/DQ2.2/DR4	+	+	+	+	-	+	+	+
DQ2.5/DQ2.2/DR4	+	+	+	+	-	-	+	+
DQ2.5/DQ2.2/DQ8	+	+	+	+	+	+	+	+

Performance characteristics

Analytical performance characteristics:

EliGene® CELIAC DQ PLUS RT kit is specific for detection of gene DQA1*05, DQB1*02, DQB1*0302 and DRB1*04. The kit detects human genomic DNA of concentration higher than 5 ng/µl added to the amplification mix.

Analytical sensitivity is 5 ng of DNA in the reaction mix.

Analytical specificity of the method is 100%. The 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 CE-IVD kits intended for the detection of analysed alleles.

Diagnostic performance characteristics:

EliGene® CELIAC DQ PLUS RT kit specificity was tested on 50 samples of human DNA with genotypes determined by CE-IVD kits intended for the detection of analysed alleles. In total, 50 samples were right determined by EliGene® CELIAC DQ PLUS RT kit.

The clinical specificity of EliGene® CELIAC DQ PLUS RT kit is 100%.

Measuring interval

The kit enables the detection of ≥ 5 ng DNA molecules in the 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 the kit depends on handling the 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

The unused content of the tube with MasterMix is stable at -20 °C. If you do not use all the volume of MasterMix, freeze it and store it at -20 °C in a dark. **Freeze/thaw tubes with MasterMix for max. 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 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 your 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 sampling contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and 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 the 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 a way as to reduce dispersion into the environment as much as possible, 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 (HLA-DQ2, HLA-DQ8/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.

In case of any problems, please contact ELISABETH PHARMACON, Ltd.

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

	Catalogue number
	The upper limit of temperature
	Batch code
	Use by (last day of the 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

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