



## 1. Intended Use

Code: KE09020

The kit "MutaGEL<sup>®</sup> HLA-DQ 2+8" allows the detection of the genetic profile determining the HLA class II serotypes DQ2 and DQ8 (MHC system). This is done by an allele-specific detection of the coding HLA-DQ2 resp. HLA-DQ8 alleles DQA1\*05, DQB1\*02 and DQA1\*03 and DQB1\*0302. For in vitro diagnostic use only.

## 2. Introduction

Coeliac disease (CD) / gluten intolerance (GI) is one of the most often chronic gastrointestinal diseases. The disease is characterised intolerance for gliadin fractions in wheat or analogous proteins in other cereals. The intake of gluten with food causes by patients chronic but reversible damages of the gastrointestinal mucous membrane which finally manifests histological in villous atrophy of the small intestine. CD/ GI is genetically strong associated with the alleles DQA1\*05 (=0501)/ DQB1\*02 (=0201 and 0202) and DQA1\*03 (=0301, 0302, =03\*\*)/ DQB1\*0302.

Endemic Sprue (ES) – in childhood called celiac disease (CD) – leads finally to villous atrophy as consequence of immune-reactions against own proteins: ES is therefore (in contrary to bacterial caused tropic sprue) an autoimmune disease developing antibodies against own body proteins (e.g. transglutaminases or the endomysium) by persons sensible for ingredients of cereals (oats in small dimension).

Sensible are all persons with the inherited specificity DQ2 and/ or DQ8 of the own-/ foreign- discrimination system HLA (= MHC), which is in case of ES are therefore present in superiority ( $\geq 95\%$  of Finish, 97% of Italian and 100% of Netherland patients) and in normal healthy persons (Europe) about 25 – 40%. This is the reason why CD/ ES is one of the most (often undetected) disease. The chronic damages of the small intestine manifest often during 6<sup>th</sup> and 18<sup>th</sup> month. The disease is and not limited exclusively to children and also extra intestinal manifestations are described.

In many patients it is possible to measure the auto-antibodies. But the analysis of the HLA-serotype DQ2 and DQ8 determining genetic profile (mutations A1\*05/ B1\*02 =DQ2 and A1\*03/ B1\*0302 =DQ8) possesses much higher sensitivity. Therefore, the PCR test MutaGEL<sup>®</sup> HLA DQ 2+8 is used for exclusion of suspicious diagnosis for GI/ ES.

*Due to the fact that presence of DQ2/ DQ8 alone is not sufficient for development of the disease it is possible to determine the number of present sensibility alleles (heterozygous or homozygous) with the aim to determine the risk for ES manifestation with DQ2/ DQ8 at all: Analysis of that gene-dose effect completes the genetic analysis and could be done with an supplementary PCR reagent set from Immundiagnostik.*

## 3. Principle of the Test

The kit „MutaGEL HLA-DQ“ contains sets of primers which amplify the DQ2-alleles DQA1\*0501, DQB1\*02 and DQ8-alleles DQA1\*03, B1\*0302. The primer sets are prepared in two separate mixes and can be used for PCR directly with the extracted sample gDNA. The resulting amplification products are subsequently identified with gelelectrophoretic methods. Additionally, an included internal control proofs (especially in absence of the analysed alleles) the correct performance of PCR in each single reaction.

## 4. Material Supplied (for 24 determinations)

▪ PCR-Mix A (DQ2)	1 x 550 µl (brown)	solution of oligonucleotides specific for DQ2 HLA-DQA1*05 + HLA-DQB1*02).
▪ PCR- Mix B (DQ8)	1 x 550 µl (blue)	solution of oligonucleotides specific for DQ8 (HLA DQA1*03 + HLA DQB1*0302).
▪ Positive control DNA	1 x 30 µl (red)	aqueous solution of human DNA with the (cloned) DNA of HLA-DQ2 gene region.
▪ Negative control	1 x 200 µl (transparent)	pure PCR water (deionized)

## 5. Materials Required but not Supplied

- DNA extraction kit (Code: KBR3005)
- PCR water (pure)
- thermal cycler + mineral oil or wax (optional for thermal cycler without heated lid)
- pipettes (0.5 - 200 µl) and sterile pipette tips (with filter)
- sterile micro tubes suitable for the thermal cycler in use
- reagents and instruments for gel electrophoresis

## 6. Storage and Stability

Store at  $\leq -18^{\circ}\text{C}$ . The reagents are stable in the unopened micro tubes until the expiration date indicated (see print on the package).

*Before use:* Spin tubes briefly before opening to collect all solutions at the bottom of the tube (contents may become dispersed during shipment).

## 7. Warning and Precautions

- For in vitro diagnostic use only.
- Test should only be performed only by skilled persons considering GLP (Good Laboratory Practice) guidelines.
- Don't use the kit after its expiration date.
- After usage, dispose all reagents and test components included in the kit in conventional garbage.
- PCR technology is extremely sensitive. The amplification of a single DNA molecule generates million identical copies. Therefore set up three separate working areas for a) sample preparation, b) PCR reagent preparation and c) DNA detection. For each working area a different set of pipettes should be reserved.
- Wear separate coats and gloves in each working area.
- Use sterile filter tips and special PCR pipettes for aerosol free pipetting.
- Routinely decontaminate your pipettes and the laboratory benches.
- Avoid aerosols (especially during opening of the reagent tubes).





## Procedure

The complete procedure is divided in three steps:

1. Sample preparation (extraction of genomic DNA).
2. Amplification with primers specific for the HLA DQ alleles.
3. Detection of the amplified DNA by gel electrophoresis.

## 8. Sample Preparation

For template use total genomic DNA which can be extracted (e. g. from 200 µl of whole blood) using commercial available DNA extraction kits according to the manufacturer's manual. Start immediately with the amplification procedure or store the extracted DNA at ≤ -18°C.

## 9. Amplification

Every set of amplifications should include a positive as well as a negative control.

For each single sample, positive control and negative control prepare the following Master Mix separately for each of the both primer mixes **A (DQ2) + B (DQ8)**; multiply the volumes necessary for each reaction with the number **N** of reactions and add 10% more volume:

PCR Reagents	Total Reaction Volume: 25 µl	Master Mix Volume
PCR Master Mix <b>A (DQ2)</b> resp. <b>B (DQ8)</b>	<b>20 µl</b>	<b>20 µl x (N+10%)</b>
<ul style="list-style-type: none"> <li>▪ aliquot <b>20 µl</b> of each prepared Master Mix <b>A</b> and <b>B</b> in <b>two different</b> PCR reaction tubes.</li> <li>▪ Samples: add <b>5 µl</b> of the <b>extracted DNA</b> (about 30 ng/ µl) to the corresponding PCR tube.</li> <li>▪ Positive control: add <b>5 µl</b> of the <b>HLA DQ2+8 positive control DNA</b> to the Master-Mix for positive references.</li> <li>▪ Negative control: add <b>5 µl</b> of PCR water to the Master-Mix for negative reference (a mixed negative control with 50% of each ready to use PCR Mix could here used to save one lane in the gel).</li> <li>▪ transfer the micro tubes into the thermal cycler and perform the following amplification protocol. Due to the high number of close related HLA genes should be used the described "<b>touch down</b>" cycling protocol (leading to high sequence specificity). In other cases, use alternative a PCR protocol with 40 cycles and the annealing temperature of <b>59°C</b> (+ do not forget the <i>hot start</i> TAQ enzyme activation step).</li> </ul>		
<b>Initial phase:</b>	95°C for 12 min (activation for <i>hot start</i> TAQ enzyme)	
<b>7 Cycles</b>	95°C for 30 sec / <b>70°C</b> for 30 sec / 72°C for 1 min	
<b>7 Cycles</b>	95°C for 30 sec / <b>65°C</b> for 30 sec / 72°C for 1 min	
<b>28 Cycles</b>	95°C for 30 sec / <b>59°C</b> for 30 sec / 72°C for 1 min	
<b>Strand Prolongation:</b>	74°C for 10 min	
<b>Final phase:</b>	10°C	

## 10. Detection of the amplified DNA and Interpretation of the Results

- Carry out a gel electrophoresis in **2 %** agarose (or 10% polyacrylamide) for about **100 Vh** (e.g. 70 min 116 volts) in 1x TBE-Buffer: mix about **15 µl** of each PCR-reaction with **4 µl** loading buffer (for each extracted DNA are two lanes necessary in order to detect the DNA fragments generated from both primer mixes (**A** and **B**) = two lanes per patient). The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard (e. g. KBR311005). The separated DNA is colored in an **ethidium bromide-** or **SybrGreen-** (5 µg/ ml) bath for about **5 - 10 min** and visualised under **UV-light (312 nm)**. The received DNA-band pattern could be (foto-) documented.
- The PCR generates for positive control (and for specific alleles, if present in the patient) for **test A (DQ2)** DNA fragments of **135 bp** (HLA-DQA1\*05) and **204 bp** (HLA-DQB1\*02). For **test B (DQ8)** DNA-fragments have sizes of **123 bp** (HLA-DQB1\*0302) and **213 bp** (HLA-DQA1\*03).
- The amplification of an additional **internal control** (human β-globin gene) leads to a DNA fragment of **400 bp**.

GENOTYPE	Length of amplicates (in base pairs)	Potential Clinical Picture
<b>DQ2 / -</b>	400 (IC) <b>204 / 135</b>	<b>GS / ES</b>
<b>- / DQ8</b>	400 (IC) <b>213 / 123</b>	<b>GS / ES</b>
<b>DQ2 / DQ8</b>	400 (IC) <b>213 / 204 / 135 / 123</b>	<b>GS / ES</b>
<b>- / -</b>	400 (IC)	<b>NO</b> with GS associated disease

The **Positiv Control DNA** possesses the genotype **HLA-DQ2 / DQ8** and the **negative control** does **not** generate any amplicate.

The **Samples** could show the **following results** (s. also table):

- **Case 1: test A** and **test B** are **both positive**, means each shows *both* specific bands (all together 4; with internal control 6). **Finding:** the sample contains one (or two) DQ2- and one (or two) DQ8- mutation associated with gluten sensitivity and endemic sprue/ celiac disease. The patient has **HLA type DQ2/ DQ8** and he **can** under viewpoint of his HLA group develop **gluten sensitivity (GS)** and **endemic Sprue (ES)**.
- **Case 2: test A** is **positive** and **test B** is **negative**, means test A has both specific DNA bands und test B has (except 400 bp control band) only one or no specific band. **Finding:** the sample contains one (or two) DQ2- mutation but no DQ8- mutation. The patient has the **HLA type DQ2** and he **can** under viewpoint of his HLA group develop **gluten sensitivity (GS)** and **endemic Sprue (ES)**.
- **Case 3: test A** is **negative** and **test B** is **positive**, means test A has (except 400 bp control band) only one or no DNA band and test B has both specific band. The patient has **HLA type DQ8** and he **can** under viewpoint of his HLA group develop **gluten sensitivity (GS)** and **endemic Sprue (ES)**.
- **Case 4: test A** und **test B** are **both negative**, means neither test A nor test B show both specific DNA bands but only each the 400 bp internal control band. **Finding:** The patient has **any other HLA type** as mentioned above and does not (with high probability: ≥ 95 % up to 100 %) **no** endemic sprue (s. introduction), means a **gluten sensitivity** can be **excluded** nearly completely.

## 11. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length and for samples at least in the internal control fragment (268 bp). If this is not the case, the sample must be tested a second time or the complete analysis must be repeated with freshly isolated DNA. If there are no positive control DNA fragments present, the amplification was incorrect and the chosen PCR conditions have to be proven/ corrected.