MutaGEL® HLA-DQ 2+8

Immundiagnostik AG, D64625 Bensheim, Stubenwald-Allee 8a, Tel: ++49-6251-70190-0, Fax: +49(0)6251-849430, **www.immundiagnostik.com**

1. Intended Use — Code: KE09020

The kit "MutaGEL® HLA-DQ 2+8" allows the **detection of the genetic profile** determining human HLA class II serotypes DQ2 and DQ8 (MHC system). This is done by detection of specific HLA-alleles coding for DQ2 resp. DQ8 (DQA1*05 + DQB1*02 and DQA1*03 + DQB1*0302) - but exclusively in the **allele combination** leading to these haplotypes (DQ2 resp. DQ8). This kit is not for single independent allele typing (and not comparable with photo-typing method). 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 villious 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 (≥ 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 6th and 18th 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® HLA DQ 2+8 is used for exclusion of suspicious diagnosis for GI/ ES.

3. Principle of the Test

The kit „MutaGEL HLA-DQ 2+8" 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 gelelctrophoretic 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**)	reagents for DQ2-specific **allele combination** (A1*05 **with** B1*02) *hot start* amplification.
▪ PCR- Mix **B** (**DQ8**)	1 x **550 µl** (**blue**)	reagents for DQ8-specific **allele combination** (A1*03 **with** B1*0302) *hot start* amplification.
▪ Positive control DNA	1 x **40 µl** (**red**)	aqueous solution of human DNA with the (cloned) DNA of HLA-DQ2 gene region
▪ Negative control	1 x **100 µl** (white)	pure PCR water (deionized)
▪ Gel loading buffer	1 x **250 µl** (transparent)	5 x concentrated loading buffer (extra heavy) with bromphenol-blue (without Xylencyanol).

5. Materials Required but not Supplied

- DNA extraction kit (e.g. MutaCLEAN® DNA Blood, Code: KBR3005)
- thermal cycler + mineral oil or PCR-wax (optional for thermal cycler without heated lid)
- pipettes (0.5 - 200 µl) and sterile pipette tips (with filter)
- sterile micro tubes (for master mix preparation) and suitable PCR tubes
- reagents and instruments for gel electrophoresis (electrophoresis buffer (TBE), chamber, power supply)

6. Storage and Stability

Store at ≤ -18°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 and 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. Routinely decontaminate your pipettes and the laboratory benches.
- Use sterile filter tips and special PCR pipettes for aerosol free pipetting. 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 both primer sets specific for the HLA DQ2- resp. DQ8- PCR (in parallel with both mixes) and **3**. Analysis of genotype by gel electrophoresis of generated amplification products.

CE

8. Sample Preparation

Use total genomic DNA as template and extract (e. g. from 200 µl of whole blood) by use of a commercial available DNA extraction kit according to the manufacturer`s manual. Start immediately with the amplification procedure (PCR) or store the extracted DNA at ≤ -18°C.

9. Amplification

Every set of amplifications should include a positive control (PC) as well as a negative control (NC).

For each single sample, PC and NC prepare the following Master Mix **separately for each** of 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 **A** (**DQ2**) resp. **B** (**DQ8**)	**20 µl**	**20 µl** x (N +10%)

- aliquot **20 µl** of (each) prepared Master Mix **A** (**DQ2**) and **B** (**DQ8**) in **two different** PCR reaction tubes.
- Samples: add **5 µl** of the **extracted DNA** (about 30 ng/ µl) into the corresponding PCR tube.
- Positive control: add **5 µl** of **HLA DQ2+8 positive control DNA** to the corresponding Master-Mix for positive control references (DQ2 resp. DQ8).
- Negative control: add **5 µl** of **PCR water** to the Master-Mix for negative reference.
- 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 "**touch down**" cycling protocol (leading to high sequence specificity). Alternative, you may also use a PCR protocol with **40** cycles and the annealing temperature of **66°C** which is somewhat less stringent (+ do not forget *hot start* Taq enzyme activation!).

Initial phase:	**95°C** for **15 min** (activation for *hot start* Taq enzyme)
5 Cycles	95°C for 30 sec / **70°C** for 30 sec / 72°C for 1 min
5 Cycles	95°C for 30 sec / **65°C** for 30 sec / 72°C for 1 min
30 Cycles	95°C for 30 sec / **59°C** for 30 sec / 72°C for 1 min
Strand prolongation	**74°C** for **10 min**
Final Phase:	**15°C** for **10 min**, resp. RT (cooling)

10. Detection of the amplified DNA and Interpretation of the Results (Analysis of Genotype)

- Carry out a gel electrophoresis in **2 %** agarose for about **100 Vh** (e.g. 70 min 116 volts) in 1x TBE-Buffer: mix about **15 µl** of each PC 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 suitable molecular-weight standards. The separated DNA is coloured in **ethidium bromide**- or **SybrGreen**- bath (5 µg/ ml) for about **5 - 10 min** and visualised under **UV light (312 nm)**. The received DNA-band pattern could be visualized (the gel loading buffer in the kit does not contain Xylencyanol which would cover the upper diagnostic relevant DNA bands) and subsequently (photo-) documented.
- The PCR generates for positive control (and in presence of corresponding HLA DQ2- resp. DQ8- **allele combination** in the samples) for **test A (DQ2)** DNA fragments of **119 bp** and **204 bp** (corresponding to allels A1*05 resp. B1*02). For **test B (DQ8)** DNA-fragments have sizes of **124 bp** and **213 bp** (corresponding to allels B1*0302 resp. A1*03). Their amplification is evaluated "positive" only if **bands of alleles** are coloured with **equal (strong) intensity** (means only **in combination** as identical stained double-band).
- **IMPORTANT**: a **positive haplotype result** (for DQ2 resp. for DQ8) is given only with simultaneous occurrence of **both** **HLA bands** from each reaction – possibly visible single bands (even when „distinct") shall not be considered as a „positive finding"!
- The amplification product of **internal control** (contained in each PCR-Mix) is **400 bp**: it is detected on principle in each PC reaction, at least in case of no further amplification products. Bit this internal control may be **weak** (or lack at all) when clinical relevant DQ2-/ DQ8- allels are present.

GENOTYPE	Length of amplificates (in base pairs)	Potential Clinical Picture
DQ2 / -	400 (IC) **204 / 119**	**GS / ES**
- / DQ8	400 (IC) **213 / 124**	**GS / ES**
DQ2 / DQ8	400 (IC) **213 / 204 / 119 / 124**	**GS / ES**
- / -	400 (IC)	**NO** with GS associated disease

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

The **Samples** could show **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- mutations 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 bands. The patient has **HLA type DQ8** and **can** under viewpoint of his HLA group develop **gluten sensitivity (GS)/endemic Sprue (ES)**.
- **Case 4**: **test A** and **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 the samples at least in the internal control fragment (400 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 been proven/ corrected. The gel-electrophoretical interpretation is suited only for the mentioned purpose and not for typing of single allels despite of DQ2-/ DQ8- question (analysis for celiac disease predisposition) because only (!) the combined occurrence of both alleles means "positive finding"!

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 • FAX: 617-419-1110

www.EagleBio.com • info@eaglebio.com

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.