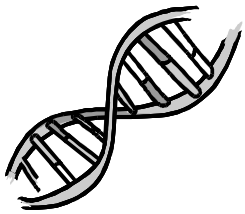
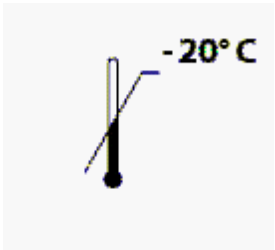


MutaPLATE® HLA DQ 2+8 (TM)

real time PCR Kit

The MutaPLATE HLA-DQ 2+8 (TM) allows the detection of the genetic profile determining the HLA class II serotypes DQ2 and DQ8 (MHC system) in real time capillary systems (e. g. LightCycler®, Roche).



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For Research Use Only.

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Content

1 Intended Use

MutaPLATE® HLA-DQ2+8 (TM) ***real time*** PCR kit is a molecular biological test for detection of the genetic profile determining the HLA class II serotypes DQ2 and DQ8 (MHC system) (LPH) in open real time PCR systems (e. g. RotorGene, SmartCycler, Light Cycler, ABI, Stratagene, Amplifa).

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 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 B1*0302 =DQ8) possesses much higher sensitivity. Therefore, the PCR test is used for exclusion of suspicious diagnosis for GI/ ES.

3 Principle of the test

MutaPLATE® HLA-DQ2+8 (TM) ***real time*** PCR Kit contains three allele specific primers and TaqMan probes, labeled with **FAM**, for the detection of the HLA-DQ2 & DQ8 alleles. The TaqMan probes bind to the amplificated target-DNA. Due to this, a fluorescence signal is generated and detected by the **optical unit** of the ***real time*** PCR instrument (e. g. Light Cycler®, Roche). Positive samples are identified due to the increase in fluorescence on the **FAM (530 nm)** channel during the PCR. An internal amplification control (IC), labeled with **Yakima Yellow (560 nm)**, is also amplified with every sample. For a valid result, there must be a successful amplification of the IC on the **YAK** channel.

4 Kit content

Each kit contains enough reagents to perform **32** tests. Each kit also contains a package insert.

Reference	Type of reagent	Volume (32 x)
Blue	enzyme mix & buffer	1300 µl
Yellow 1	primer / probe mix DQA1*05	210 µl
Yellow 2	primer / probe mix DQB1*02	210 µl
Yellow 3	primer / probe mix DQB1*0302	210 µl
White	primer / probe mix amplification control	3 x 158 µl
Red 1	positive control DQA1*05/DQB1*02	30 µl
Red 2	positive control DQB1*0302	10 µl
Green	negative control	50 µl

5 Required materials

Provided:

- Reagents for ***real time*** PCR
- Package insert

Not provided:

- ***real time*** PCR system (e. g. RotorGene)
- PCR reaction tubes or plates
- Cryo container for PCR reaction tubes
- DNA extraktion kit for isolation of genomic DNA (ca. 10 ng/µl), e.g. **MutaCLEAN® DNA Blood, KG1033, Immundiagnostik**
- Pipetts (0,5 – 200 µl) with sterile filter Tipps for micro pipets
- sterile microtubes
- gloves (powder free)

6 Storage and handling

- All reagents should be **stored at <-20°C till immediate use**. Spin down kit components in their vials before long-term storage.
- **Avoid several freeze / thaw** cycles for the reagents (if necessary prepare suited aliquots and freeze again **immediately**).
- During preparation of PCR perform all working steps in a cryo-container or **cool all reagents** in suited manner.
- Primer-/ Probe-Mix should be **stored in the dark** (**light protection**).
- All reagents can be used until the expiration date (printed on the labels).

7 Warnings and precautions

- For research use only.
- This assay needs to be carried out by especially in molecular biology skilled personnel: This assay needs to be run according to GLP (Good Laboratory Practice).
- Clinical samples should be regarded as potentially infectious materials.
- Mix all reagents carefully before use, but do not vortex.
- Do not use the kit after its expiration date.

8 Test procedure

Before start, **decontaminate** all working areas and used instruments. Thaw kit components **gently at 5 - 8°C** and handle detection mixes in the dark. Prepare the necessary amount of PCR reaction tubes in a pre-cooled cooling block and consider additional 2 tubes for the controls. Keep DNA samples ready and mix well before use.

Enzyme mix (***ready to use***)

This ready to use enzyme mix can be thawed twice at 5 - 8°C, provided that it was not stored longer than one hour (cooled) during the working steps.

Master mix preparation DQA1*05

Following table shows the composition for **one reaction** (final volume: 25 µl). For analysis of several samples in parallel, a **master mix** should be prepared in a sterile vial **multiplying** each single volume by the number **N** of samples (incl. controls). ***Additionally, 10% more volume should be calculated for reasons of inaccuracy***. The reagents should be pipetted in same order as indicated in the table:

Reagent	Volume	Master Mix Volume
Detection Mix (yellow)	**6 µl**	**6 µl** x (N + 10%)
Detection Mix (**white**)	**4.5 µl**	**4.5 µl** x (N + 10%)
Enzyme Mix ***ready to use*** (**blue**)	**12.5 µl**	**12.5 µl** x (N + 10%)

Master mix preparation DQB1*02

Following table shows the composition for **one reaction** (final volume: 25 µl). For analysis of several samples in parallel, a **master mix** should be prepared in a sterile vial **multiplying** each single volume by the number **N** of samples (incl. controls). ***Additionally, 10% more volume should be calculated for reasons of inaccuracy***. The reagents should be pipetted in same order as indicated in the table:

Reagent	Volume	Master Mix Volume
Detection Mix (yellow)	**6 µl**	**6 µl** x (N + 10%)
Detection Mix (**white**)	**4.5 µl**	**4.5 µl** x (N + 10%)
Enzyme Mix ***ready to use*** (**blue**)	**12.5 µl**	**12.5 µl** x (N + 10%)

Master mix preparation DQB1*0302

Following table shows the composition for **one reaction** (final volume: 25 µl). For analysis of several samples in parallel, a **master mix** should be prepared in a sterile vial **multiplying** each single volume by the number **N** of samples (incl. controls). ***Additionally, 10% more volume should be calculated for reasons of inaccuracy***. The reagents should be pipetted in same order as indicated in the table:

Reagent	Volume	Master Mix Volume
Detection Mix (yellow)	**6 µl**	**6 µl** x (N + 10%)
Detection Mix (**white**)	**4.5 µl**	**4.5 µl** x (N + 10%)
Enzyme Mix ***ready to use*** (**blue**)	**12.5 µl**	**12.5 µl** x (N + 10%)

Mix prepared master mix well by gently pipetting (about **15 – 20 x, do not vortex**) and aliquot **23 µl** into each PCR reaction tube.

Samples

Add **2 µl** of each sample DNA in the corresponding PCR reaction tube; use first **both controls** (1. negative control, 2 µl and 2. positive control, 2 µl). Close the tubes and transfer them into the real time PCR instrument (keep position of samples).

Protocol

Activate following **PCR-protocol** and perform subsequently the ***real time*** PCR:

Experimental Protocol

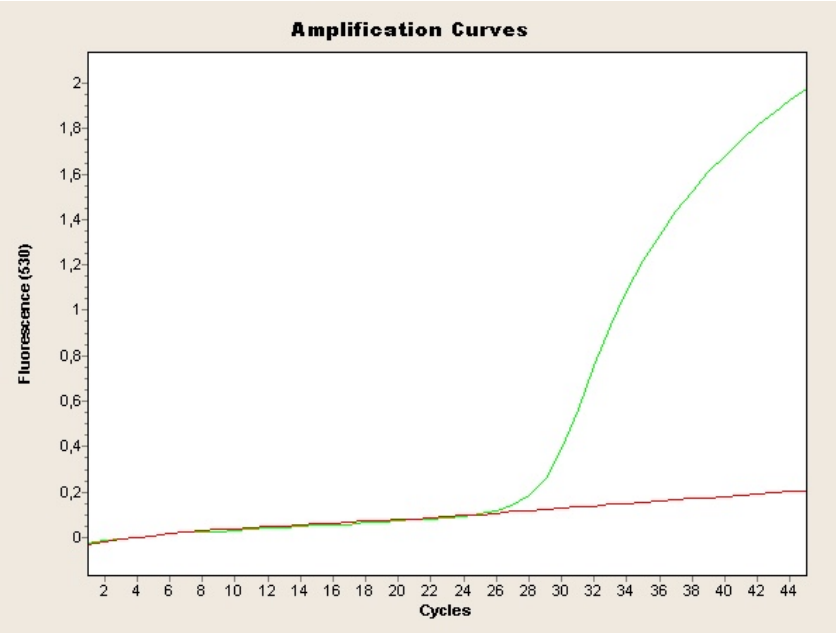
Program:	**Denaturation**		1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	94	120	None

Program:	**Amplifikation**		45
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	94	10	None
2	58	30	**Single**
3	72	30	None
Program:	**Cooling**		1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Acquisition Mode
1	40	30	None

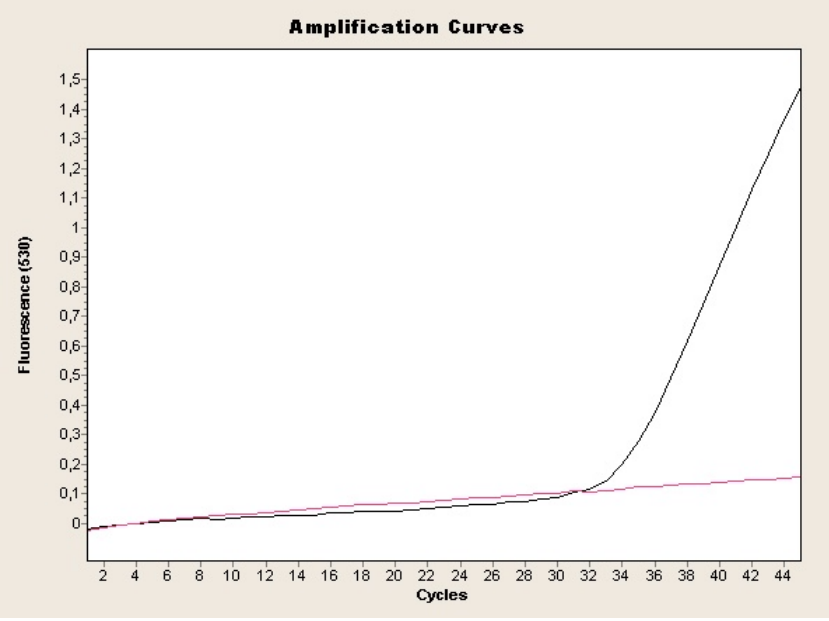
9 Analysis of genotype and interpretation of results

The provided controls contain templates, which are positive for the alleles HLA-DQ2 (DQA1*05 and DQB1*02) and HLA-DQ8 (DQB1*0302). The results of the ***real time*** PCR are measured at **510-530 nm**. The amplification control are measured at **550-560 nm**. The following diagrams show typical examples of positive and negative samples. A **color compansation file** might be required on some ***real time*** PCR devices, e.g. LightCycler 2.0.

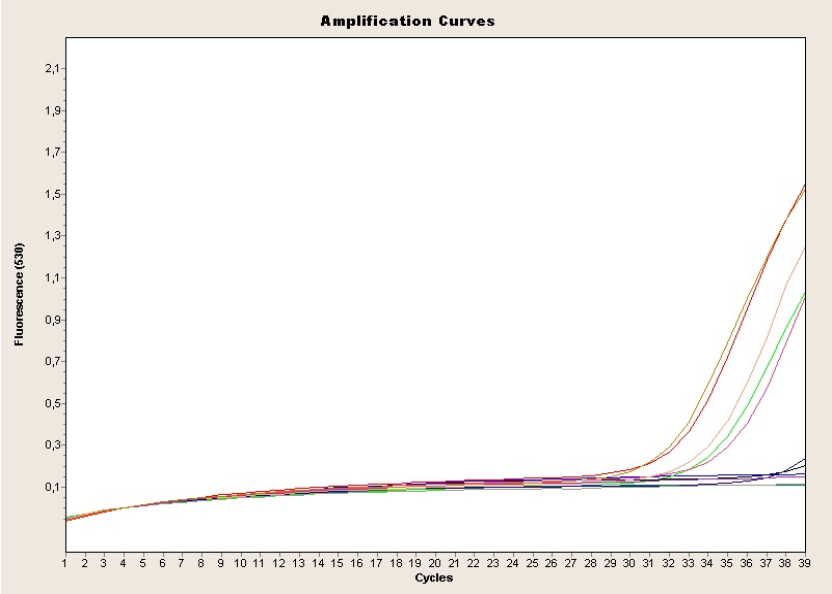
HLA-DQA1*05 at 530 nm



HLA-DQB1*2 at 530 nm

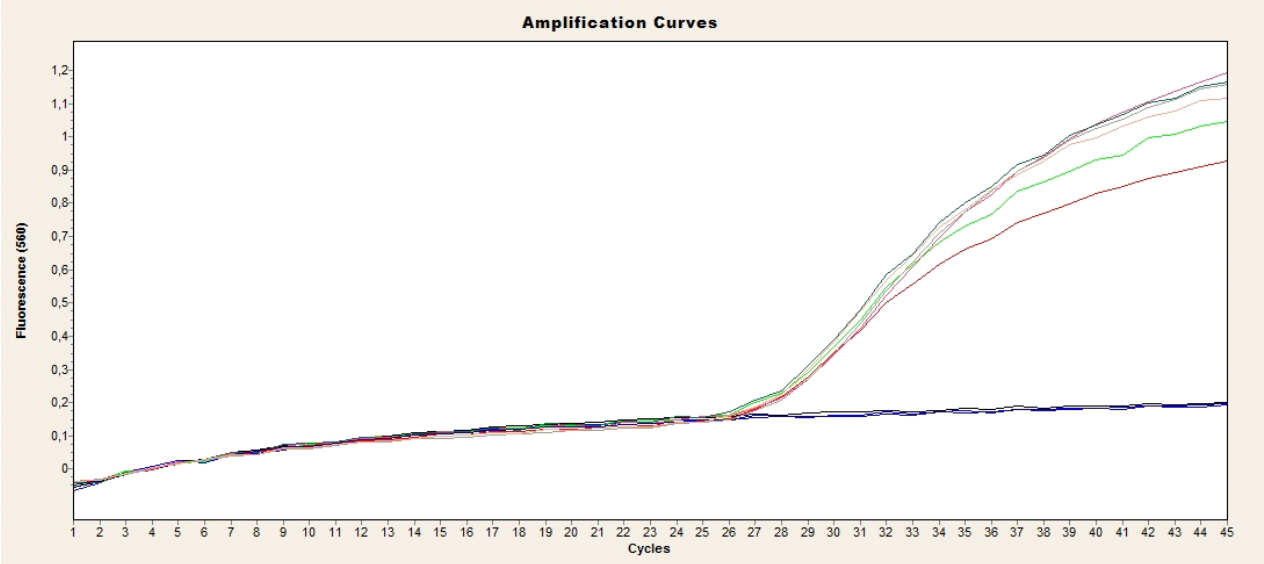


DQB1*0302 at 530 nm



Positive samples for DQB1*0302 have crossing points (cp) < 35 cycles. Samples with a cp > 35 cycles or no increase in fluorescent signal are negative.

Amplification control at 560 nm



10 Troubleshooting

No fluorescence peak with positive control or samples at about **510-530 nm** or **550-560 nm** respectively:

- Proof PCR-program of the real time PCR instrument in use:

⇒ repeat analysis with corrected protocol.

- **MutaPLATE® HLA-DQ2+8 (TM)** kit was thawed/ frozen more than twice or stored longer than four days at 2-8 °C:

⇒ consider storage recommendations. Repeat analysis with new **MutaPLATE® HLA-DQ2+8 (TM)** reagents.

- low quality of DNA -template:

⇒ exactly follow the manufacturer`s manual for DNA extraction.

Low fluorescence peak at about **510 - 530 nm** or **550 - 560 nm** respectively:

- mix single components carfully before use (only by pipetting several times - do not vortex!).
- cool all stock solutions during the working steps in suited manner and protect the detection mix from light.
- Working on ice or with cooled (4°C) block is recommended.

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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.

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