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MutaPLATE® HLA DQ 2+8 (TM)

Real-Time-PCR Kit

For the analysis of the HLA-alleles DQA1*05, DQB1*02 und DQB1*03:02/*03:05

Valid from 2022-02-23

REF **KF190532**
KF190596

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1 INTENDED USE

The MutaPLATE® HLA DQ 2+8 (TM) Real-Time PCR Kit is a molecular biological assay for the detection of the HLA alleles DQA1*05, DQB1*02[1] and DQB1*03:02/*03:05[2,3] based on the TaqMan-technology.

[1] The number of copies of DQB1*02 is not determined by this method.

[2] Due to sequence homologies this method cannot differentiate between DQB1*03:02 and DQB1*03:05. However, the frequency of DQB1*03:05 is very low, about 0.4 % in the Caucasian population, whereas DQB1*03:02 has a frequency of about 15 % (http://www.allelefrequencies.net, Nov. 2018). Worldwide the frequency of DQB1*03:05 is 0.07 % and the frequency of DQB1*03:02 is 11.5 % (Solberg et al., Hum Immunol, 2008).

[3] The detection of other very rare alleles cannot be completely excluded.

2 INTRODUCTION

Celiac disease / gluten intolerance is one of the most frequent chronic gastrointestinal diseases. It is a genetic disease, in which the body is not able to process the gluten present in many cereals. Almost all celiac disease patients are carrier of HLA-DQ2 (HLADQA1*05 and HLA-DQB1*02) or HLA-DQ8 (HLA-DQB1*03:02, often in combination with DQA1*03). If DQ2 and DQ8 are not detected, celiac disease can be excluded with a probability of over 95 %.

3 PRINCIPLE OF THE TEST

The MutaPLATE® HLA DQ 2+8 (TM) Real-Time PCR Kit contains two sequence specific primers flanking the region of interest and two TaqMan probes specific to the region containing the mutation. The two TaqMan probes are labeled at the 5' end with different fluorophores (reporter dyes) which are used for the allelic discrimination. On the 3' end the TaqMan probes are labeled with a non-fluorescent quencher. The proximity of the reporter dye to the quencher inhibits the fluorescence of the reporter molecule. During amplification the probes hybridize specifically to the DNA fragments. The 5' nuclease activity of the polymerase cleaves the hybridized probes releasing the reporter from the quencher generating a fluorescent signal.

4 PACKAGE CONTENTS

The components supplied are sufficient for the preparation of 32 (KF190532) or 96 (KF190596) reactions.

Table 1: Components of the MutaPLATE® HLA DQ 2+8 (TM) Real-Time-PCR Kit .

Label	Lid Colour	Content	
		32	96
Enzyme mix	blue	1 x 1313 µl	2 x 1970 µl
Detection mix 1 (DQA1*05)	yellow	1 x 210 µl	1 x 630 µl
Detection mix 2 (DQB1*02)	brown	1 x 210 µl	1 x 630 µl
Detection mix 3 (DQB1*03:02)	purple	1 x 210 µl	1 x 630 µl
Detection mix IC	white	1 x 473 µl	1 x 1419 µl
Positive control 1 (DQA1*05 / DQB1*02)	red	1 x 30 µl	1 x 990 µl
Positive control 2 (DQB1*03:02)	orange	1 x 15 µl	1 x 45 µl
Negative control	green	1 x 150 µl	1 x 150 µl

5 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- DNA extraction kit (e.g. MutaCLEAN® Universal RNA/DNA, KG1038)
- Roche LightCycler® 2.0 real-time PCR system
  - *The CE conformity is only given with this instrument.*
- Roche LightCycler® capillaries
- Roche LightCycler® Cooling Block
  - **Or:** Open real-time PCR system (with plates/stripes or tubes)
  - Sterile PCR reaction tubes or 96-well plate/stripes (white)
  - Sterile reaction tubes
- Calibrated pipettes (variable volumes) and sterile disposable tips with filter
- Optional: Liquid handling system for automation

6 TRANSPORT, STORAGE AND STABILITY

The MutaPLATE® HLA DQ 2+8 (TM) real-time PCR kit is transported frozen on dry ice or cool packs. All components are to be stored protected from light at a minimum of -20 °C immediately after receipt. Avoid multiple freeze-thaw cycles (make aliqouts if necessary). Do not use after the expiry date indicated on the package.

Be sure to protect the detection mixes from direct sunlight during the entire test procedure.

7 WARNINGS AND PRECAUTIONS

Read the instructions for use carefully before using the product.

- All samples must be considered potentially infectious and/or biohazardous and all items that come into contact with the specimens must be considered potentially contaminated.
- Real-time PCR must be performed in laboratories suitable for this purpose and by specially trained personnel.
- The assay must always be carried out according to the instructions supplied with the kit.
- Areas for sample preparation and preparation of the PCR master mix should be strictly separated.
- Pipettes, tubes and other working materials must not circulate from one area to the other.
- Always use pipette tips with filters.
- Always wear powder-free disposable gloves when using the kit
- Clean pipettes and work surfaces regularly with suitable decontamination solution (no ethanol-containing agents).
- Contamination of eluates and kit components with microbes or nucleases (RNAs and DNAses) should be avoided.
- Positive and potentially positive material must be kept separate from all other kit components at all times.
- Do not open reaction tubes/plates after amplification in order to avoid contamination.
- In accordance with guidelines or requirements of local, state or federal regulations or authorised organisations, additional controls may be tested.
- Do not autoclave reaction tubes after PCR as this will not degrade the amplified nucleic acid and risks contaminating the laboratory area.
- Dispose of samples and test waste according to your local safety regulations.
- Refrigerate all PCR reagents while working.
- The purity (A260/A280) of the genomic DNA should be between 1.8 and 2.0.

8 SAMPLE MATERIAL

Starting material for the MutaPLATE® HLA DQ 2+8 (TM) real-time PCR kit is genomic DNA isolated from clinical samples (blood) using a suitable extraction kit.

9 REAL-TIME-PCR

9.1 Important points before starting

- Please pay attention to chapter 7 “Warnings and precautions”.

- Before setting up the Real-Time-PCR familiarise yourself with the Real-Time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the PCR set up.
- In every PCR run all Positive Controls and one Negative Control should be included.
- Before each use, all reagents must be gently thawed, thoroughly mixed (do not vortex) and briefly centrifuged.
- Protect the detection mixes from exposure to light.
- We recommend always cooling the reagents and the preparation in a cooling block (+4 to +8 °C) or on ice while working.

9.2 Procedure

For amplification, two reaction tubes are required per sample and two additional reaction tubes per master mix are required for the negative and the positive control. The following tables show the volumes to be pipetted per sample. For the analysis it is recommended to prepare a master mix for the number of samples (incl. negative and positive control) (N) plus 10 % to compensate for inaccuracies. The master mixes are pipetted as described in tables 2, 3 and 4:

Master mix 1 (DQA1*05)

Table 2: Preparation of master mix 1 (DQA1*05)

Reagent	Volume per 25 µl - reaction mix	Master mix volume
Detection mix 1 (**yellow**)	6 µl	6 µl * (N + (N * 0.1))
Detection mix IC (**white**)	4.5 µl	4.5 µl * (N + (N * 0.1))
Enzyme mix (**blue**)	12.5 µl	12.5 µl * (N + (N * 0.1))

- Mix the Master Mix carefully by pipetting up and down or by inverting and centrifuge briefly.
- Add **23 µl** of the Master Mix to each capillary.
- For the negative control add **2 µl** of the supplied negative control (**green**).
- For the positive control add **2 µl** of the supplied positive control 1 (**red**).
- For each sample to be analysed, add **2 µl** of the extracted genomic DNA to the corresponding capillary.

Master mix 2 (DQB1*02)

Table 3: Preparation of master mix 2 (DQB1*02)

Reagent	Volume per 25 µl - reaction mix	Master mix volume
Detection mix 2 (**brown**)	6 µl	6 µl * (N + (N * 0.1))
Detection mix IC (**white**)	4.5 µl	4.5 µl * (N + (N * 0.1))
Enzyme mix (**blue**)	12.5 µl	12.5 µl * (N + (N * 0.1))

- Mix the Master Mix carefully by pipetting up and down or by inverting and centrifuge briefly.
- Add **23 µl** of the Master Mix to each capillary.
- For the negative control add **2 µl** of the supplied negative control (**green**).
- For the positive control add **2 µl** of the supplied positive control 1 (**red**).
- For each sample to be analysed, add **2 µl** of the extracted genomic DNA to the corresponding capillary.

Master mix 3 (DQB1*02)

Table 4: Preparation of master mix 2 (DQB1*02)

Reagent	Volume per 25 µl - reaction mix	Master mix volume
Detection mix 3 (**purple**)	6 µl	6 µl * (N + (N * 0.1))
Detection mix IC (**white**)	4.5 µl	4.5 µl * (N + (N * 0.1))
Enzyme mix (**blue**)	12.5 µl	12.5 µl * (N + (N * 0.1))

- Mix the Master Mix carefully by pipetting up and down or by inverting and centrifuge briefly.
- Add **23 µl** of the Master Mix to each capillary.
- For the negative control add **2 µl** of the supplied negative control (**green**).
- For the positive control add **2 µl** of the supplied positive control 2 (**orange**).
- For each sample to be analysed, add **2 µl** of the extracted genomic DNA to the corresponding capillary.

Close the capillaries with the corresponding lids, transfer them into the LightCycler® carousel and spin them down in the LightCycler® centrifuge (if a table top centrifuge is used, spin the capillaries in the adapters of the cooling block at 3000 rpm for 15 s).

Subsequently place the carousel in the LightCycler® and use the PCR protocol described in 9.3.

9.3 Instrument settings

For the Real-Time-PCR use the thermal profile shown in table 5.

Table 5: Real-Time-PCR thermal profile

Description	Time	Temperature	Ramp rate	Cycles	Acquisition
Initial Denaturation	120 s	94 °C	max.	1	none
Denaturation	10 s	94 °C	max.	45	none
Primer annealing and elongation	50 s	60 °C	max.		**single**
Cooling	30 s	40 °C	max.	1	-

10 DATA ANALYSIS

The HLA DQ 2+8 PCR Real-Time kit detects the presence of the HLA alleles DQA1*05, DQB1*02 and DQB1*03:02. The corresponding TaqMan probe for the three alleles is labeled with FAM (**510 nm**, **green**). If no HLA allele is present, no amplification occurs and therefore no fluorescence of the FAM-labeled TaqMan probe is detected. To ensure in this case that the PCR was correctly carried out and an internal amplification control (IC) is included into the PCR. The TaqMan probe for the internal amplification control is labeled with YAK (**555 nm**, **yellow**) and should always give a signal.

Corresponding to the genotype the following results can be achieved:

1. HLA allele present:

Increase of the fluorescence signal of the **FAM**-labeled TaqMan probe and increase of the fluorescence signal of the **YAK**-labeled TaqMan probe.

2. HLA allele is not present:

No increase of the fluorescence signal of the **FAM**-labeled TaqMan probe and increase of the fluorescence signal of the **YAK**-labeled TaqMan probe.

The evaluation of the amplification curves (determination of the crossing points) is done by adding an analysis of the type “absolute quantification”. The results of the analysis for the HLA alleles DQA1*05, DQB1*02 and DQB1*03:02 are analysed at **510 - 530 nm** / **green** and for the IC at **550 - 570 nm** / **yellow**. Please use a corresponding color compensation file.

The following figures show the representative results for the detection of the HLA allele **DQA1*05**: **green curve** - negative control, **dark blue curve** - DQA1*05 not detected, **blue curve** - DQA1*05 detected.

FAM (510 - 530 nm / green) - detection of the allele DQA1*05

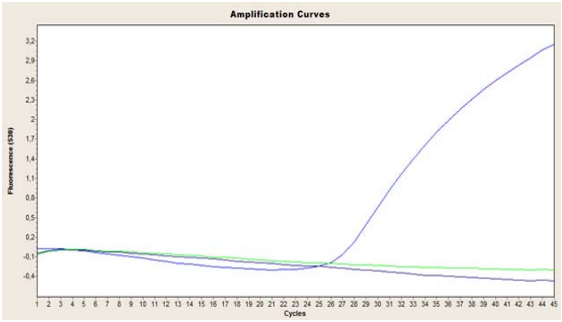

Fig. 1: Evaluation of HLA DQA1*05

YAK (550 - 570 nm / yellow) - internal amplification control

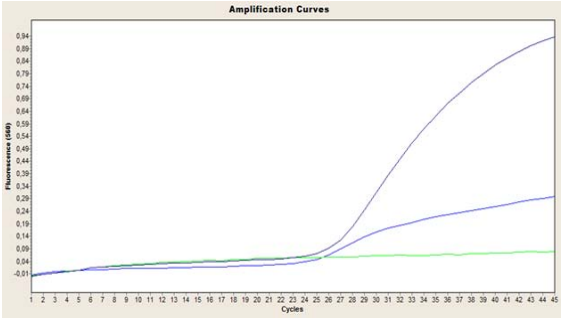

Fig. 2: Evaluation of HLA DQA1*05 - internal amplification control

The following figure shows the representative results for the detection of the HLA allele **DQB1*02**: **grey curve** - negative control, **violet curve** - DQB1*02 not detected, **dark blue curve** - DQB1*02 detected.

FAM (510 - 530 nm / green) - detection of the allele DQB1*02

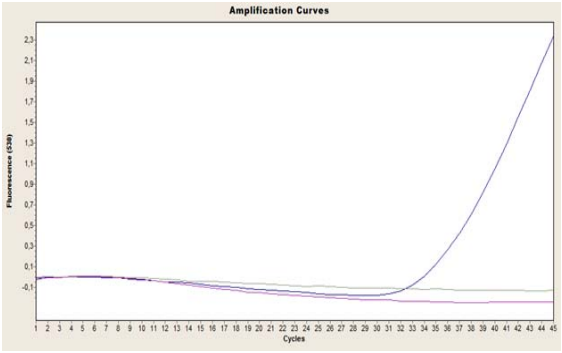

Fig. 3: Evaluation of HLA DQB1*02

YAK (550 - 570 nm / yellow) - internal amplification control

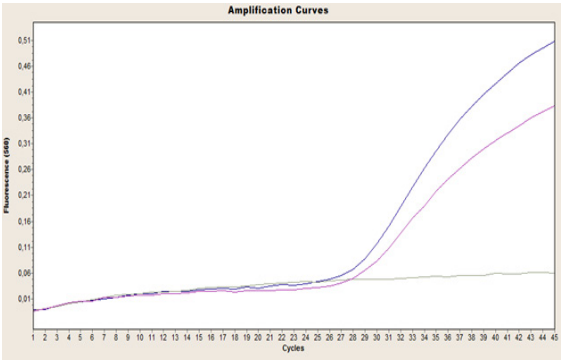

Fig. 4: Evaluation of HLA DQB1*02 - internal amplification control

The following figure shows the representative results for the detection of the HLA allele **DQB1*03:02**: **red curve** - negative control, **turquoise curve** - DQB1*03:02 not detected, **blue curve** - DQB1*03:02 detected.

FAM (510 - 530 nm / green) - detection of the allele DQB1*03:02

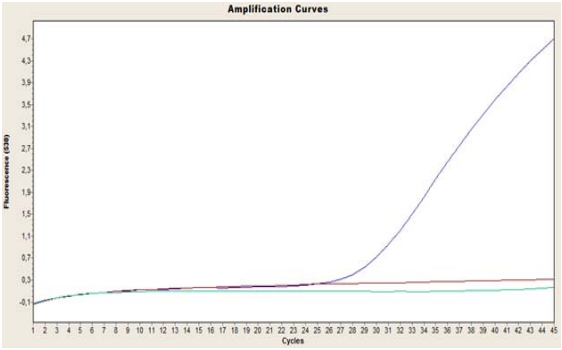

Fig. 5: Evaluation of HLA DQB1*03:02

YAK (550 - 570 nm / yellow) - internal amplification control

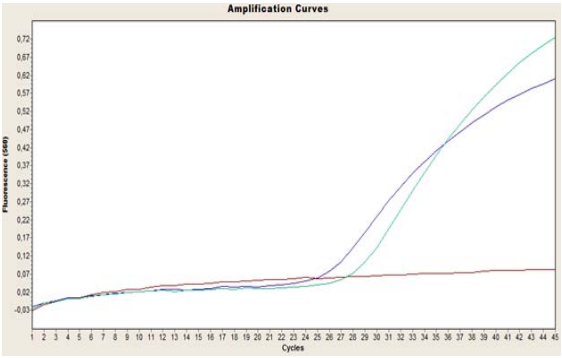

Fig. 6: Evaluation of HLA DQB1*03:02 - internal amplification control

The provided Positive Control 1 (**red**) contains a template, which is positive for the alleles DQA1*05 and DQB1*02. The provided Positive Control 2 (**orange**) contains a template, which is positive for the allele DQB1*03:02.

11 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time-PCR. If you have further questions, please do not hesitate to contact our scientists on info@immundiagnostik.com.

No fluorescence peak in the positive control or samples at about 510 - 530 nm or 550 - 560 nm.

Check the PCR programme of the real-time PCR system and repeat the analysis with the corrected protocol.

Detection mixes have been subjected to more than two freeze cycles or have been stored at 2-8 °C for more than four days. Repeat the analysis with a fresh aliquot or new detection mix.

The quality of the starting DNA is not sufficient. Use freshly extracted DNA and determine the concentration/purity before use.

The detection mixes were not protected from light exposure. Repeat the analysis with a fresh aliquot or new PCR reagents.

Low flourescence peak at about 510 - 530 nm or 555-560 nm

Mix individual components carefully before use (only by pipetting several times - do not vortex!).

Cool all stock solutions appropriately during the working steps and protect the detection mixes from light irradiation.

Work on ice or with a cooled block (4 °C).

12 LIMITATIONS OF THE METHOD

The result is provided to the treating physician as supporting material and should never be used exclusively for diagnosis or treatment recommendations. The diagnosis as well as the treatment decisions to be taken remain the full responsibility of the physician.

The accuracy of genetic tests is not 100 %. However, it has been found to be over 98 % accurate based on validation data for this test. Furthermore, genetic test results must be considered in the context of the patient's clinical representation and known familial risks in the patient's environment.

The test only analyses a selection of markers. Therefore, a negative test result of the patient does not completely exclude a risk of any kind.

13 ABBREVIATIONS AND SYMBOLS

DNA	Deoxyribonucleic acid	REF	Catalog number
PCR	Polymerase chain reaction	→REF	To be used with
ENZYME MIX	Enzyme mix	Σ	Contains sufficient for <n> test
DETECTION MIX 1	Detection mix 1		Upper limit of temperature
DETECTION MIX 2	Detection mix 2		Manufacturer
DETECTION MIX 3	Detection mix 3	LOT	Lot number
DETETCTION MIX IC	Detection mix IC		Consult instructions for use
CONTROL 1 +	Positive control 1		Use by YYYY-MM-DD
CONTROL 2 +	Positive control 2	IVD	*In vitro* diagnostic medical device
CONTROL –	Negative control	CONTENT	Content

14 LITERATURE

1. Solberg et al., Hum Immunol, 2008