EN

INSTRUCTIONS FOR USE

FastQ® CD

Test kit for determination of HLA-DQ alleles associated with coeliac disease on a molecular genetic basis

Electronic instructions for use see www.bag-diagnostics.com

CE IVD

REF 728202 FastQ® CD

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If a complete chapter is new or changed, only the headline is marked in orange.

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CONTENTS

1 INTENDED USE

The intended purpose of the FastQ® CD kit is the molecular genetic determination of the presence of the following HLA-DQ alleles that are associated with coeliac disease:

- DQA1*02:01, DQA1*05:01, DQA1*05:05
- DQB1*02:01, DQB1*02:02, DQB1*03:02

2 PRODUCT DESCRIPTION

Coeliac disease (CD), is a chronic inflammatory disorder of the small intestine caused by an inappropriate immune response to dietary gluten proteins of wheat, rye, and barley. In diagnosis of CD four aspects are considered: symptoms, celiac antibodies in the serum (TG2-IgA), duodenal histology and the presence of HLA-DQ2 and/or DQ8. Coeliac disease is one of the diseases with the strongest HLA association (Sollid, L M et al. 1989, Sollid L M, and E Thorsby, 1990). The diagnostic value of this parameter is based on its negative predictive value, especially in patients who are sero-negative despite histological changes or in cases where there was no serological confirmation at the time of CD diagnosis (Husby et al. 2019). This strong HLA association applies to defined heterodimers of HLA-DQA and DQB antigens. The strongest association (90-95% CD) exists with DQ2.5 (DQA1*05:01, DQB1*02:01) and weaker associations (5-10% CD) with DQ8 (DQA1*03, DQB1*03:02) and DQ2.2 (DQA1*02:01, DQB1*02:02) (Sollid and Lie 2005). In a small group of patients who do not carry DQ2.5, DQ2.2 or DQ8, almost all are DQ7.5 (DQA1*05, DQB1*03:01) (Karell et al. 2003, Bergseng, Elin et al. 2015).

The detection of the presence or absence of HLA-DQ2 and/or DQ8 is an essential laboratory test for the exclusion of CD, in addition to the serological differential diagnostic procedures (anti-TG2 and/or anti-EMA). In patients with a negative test result, coeliac disease can be ruled out with a high degree of certainty. The detection of HLA-DQ2 and/or DQ8 is part of the algorithm for diagnosis of coeliac disease (see Figure 1 below, Al-Toma et al., 2019).

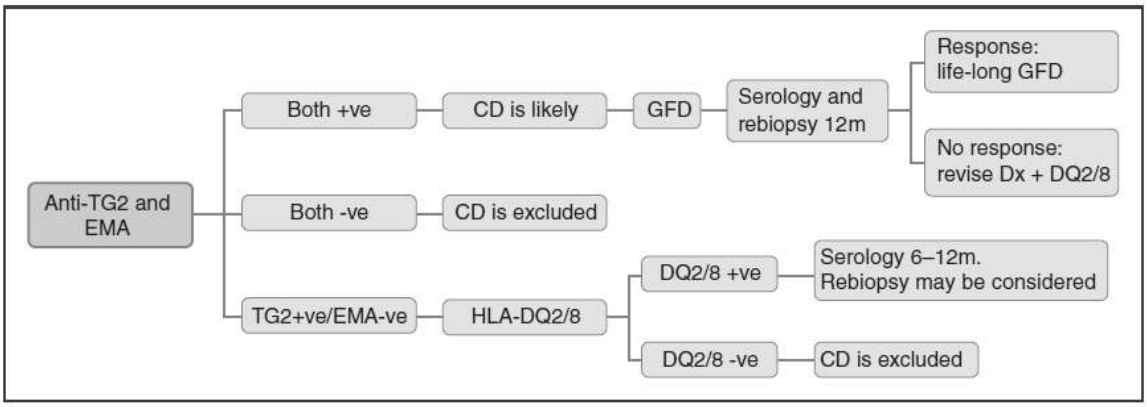


Figure 1: Algorithm for diagnosis of coeliac disease. Note that according to the current guideline, the differential diagnosis of CD is only made via HLA-DQ2/DQ8 if the serological findings are uncertain. (GFD = gluten free diet) – from Al-Toma, Abdulbaqi et al. "European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders." United European gastroenterology journal vol. 7,5 (2019): 583-613. doi:10.1177/2050640619844125

3 TEST PRINCIPLE

The test is performed with genomic DNA as starting material. The DNA is amplified in a PCR with sequence-specific primers (SSP). The primers were specially developed for the selective amplification of specific parts of the HLA-DQ gene. The amplicons are detected with likewise gene locus specific fluorescent dye-labelled hydrolysis probes (TaqMan® probes), which increases the specificity of the test compared to a conventional SSP.

If amplicons are present, the probes are hydrolyzed by the Taq polymerase and a fluorescence signal is generated that increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR-Cycler.

The test is performed with two PCR mixes that detect the internal positive control (*human growth hormon*) and the disease-associated haplotypes with different fluorescent colours.

4 MATERIAL

4.1 Contents of the FastQ® CD kit (48 Tests)

- **130 µl Q Primermix CD I**, ready to use, contains primers and probes
- **130 µl Q Primermix CD II**, ready to use, contains primers and probes
- **260 µl Plex Mix**, ready to use, contains dNTPs, Taq Polymerase, reaction buffer (contains the hazardous substance 2-methylisothiazol-3(2H)-one at a concentration of < 0.05%, see chapter 8 and 14)
- **Electronic instructions for use / kit file**, available from the download server www.service.bag-diagnostics.com, for further information see accompanying information sheet in the kit.

4.2 Additionally required reagents and devices

- Reagents for DNA isolation (validated DNA isolation kits see 6.2)
- Real-Time PCR-Cycler (validated cyclers see 4.3)
- RT-PCR reaction tubes with caps or foils (validated products see 4.3)
- Aqua dest. (DNAse free)
- Piston pipettes (0,5 – 1000 µl) and tips
- Centrifuge (e.g. PlateFuge – MicroCentrifuge by Benchmark Scientific)
- Colour Compensation kit for LightCycler® 480 II (REF **728259 RT CC Universal LC480**) provided by BAG Diagnostics)

4.3 Validated cyclers and reaction tubes

Cycler	RT-PCR reaction tubes	RT-PCR closing systems
CFX96™ / CFX Opus 96 Real-Time PCR Detection System, Comp. Bio-Rad	FrameStar® Break-A-Way PCR Plate 96 white wells, black frame Product No. 4ti-1201, Comp. Azenta Life Sciences	Crystal Strips Product No. 4ti-0755, Comp. Azenta Life Sciences qPCR Seal (Optically clear adhesive film) Product No. 4ti-0560, Comp. Azenta Life Sciences
LightCycler® 480 II Real-Time PCR Detection System, Comp. Roche Molecular Systems Inc.	Roche Multiwell Plate 96, white Product No. 04729692001, Comp. Roche	qPCR Seal (Optically clear adhesive film) Product No. 4ti-0560 Comp. Azenta Life Sciences
QuantStudio™ 6 Flex System, Applied Biosystems / Thermo Fisher Scientific	Removable 8 Well PCR Tube Strip, Product No. 4ti-0753, Comp. Azenta Life Sciences	Crystal Strips, Product No. 4ti-0755, Comp. Azenta Life Sciences

Note: If other realtime cyclers, reaction tubes and closing systems are used they must be validated by the user.

5 STORAGE AND STABILITY

The kits are shipped on dry ice. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The freeze-thaw cycle testing has shown that up to 12 cycles for the Plex Mix and up to 15 cycles for the Q Primermixes has no detrimental effects on the quality of the kit. No data are available yet for more cycles. Therefore, it is recommended to aliquot the reagents if necessary.

If the protective packaging is damaged, please contact the customer service.

Thaw the Q Primermixes and the Pley Mix directly before preparing the PCR. Immediately place the prepared PCR plates/strips in the real-time thermal cycler and start the PCR run.

6 TEST PROCEDURE

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques. The results of these tests must not be used as sole basis for diagnotical and/or clinical decisions.

Special safety conditions must be observed in order to avoid contamination and thus false reactions:

- Wear gloves during work (powder-free, if possible).
- Use new tips with each pipetting step (with integrated filter).
- If possible, use separate working areas for pre-work (DNA isolation and PCR set up) and post-amplification (detection and PCR).
- Use devices and other materials only at the respective places and do not exchange them.

6.2 DNA Isolation

The sample material for the isolation of genomic DNA must be sent in appropriate blood collection systems. For the test EDTA or Citrate blood is required. The presence of heparin potentially inhibits PCR; therefore blood collection systems with heparin are not suitable (Beutler et al. 1990) and must not be used.

It is recommended to use CE IvD certified kits for the DNA isolation.

Validated DNA isolation kits:

- Qiagen QIAamp DNA Blood Kits (columns)

If the established standard method of the lab is used for gDNA isolation and this is not the validated kit above, it must be validated by the user.

A DNA concentration of 10 - 50 ng/µl is required to perform the **FastQ® CD** test.

The DNA must have the following purity indexes:

- OD_{260}/OD_{280} = > 1.5 and < 2.0
  Higher values are an indicator for contamination with RNA, lower values for a contamination with proteins.
- OD_{260}/OD_{230} = > 1.8
  Lower values indicate a contamination with salt, carbohydrate or organic solvents.

6.3 Amplification

Reaction tubes recommended by the manufacturer of the real-time cycler or the materials recommended in chapter 4.3 should be used.

For each sample the following reagents are pipetted into a reaction tube:

Reaction I		Reaction II	
2 µl	Q Primermix CD I	**2 µl**	Q Primermix CD II
2 µl	Plex Mix	**2 µl**	Plex Mix
1 µl	Sample DNA (10-50 ng/µl)	**1 µl**	Sample DNA (10-50 ng/µl)
5 µl	Aqua dest. (DNAse free)	**5 µl**	Aqua dest. (DNAse free)

The reaction volume for each RT-PCR test is 10 µl.

Note: Vertical alternating plate loading of the sample with Primermix CD I and II is obligatory for evaluation with the PlexTyper® software.

If a premix of Q Primermix, Plex Mix and Aqua dest. is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

If a **negative control (NTC)** should be performed prepare a PCR reaction with Aqua dest. instead of DNA.

Close the reaction tubes and briefly spin down the liquid. Ensure that no bubbles are present in the wells. If bubbles are observed, gently tap assay on the bench to remove the bubbles.

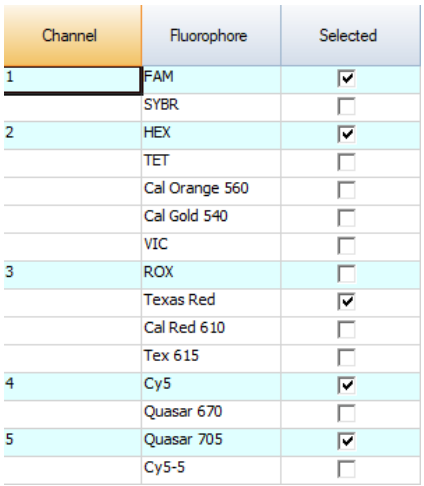
6.4 Setup of the RT-PCR cycler

The following fluorophores are used for the **FastQ® CD** kit:

Fluorophor	Wave lenght in nm	
FAM	Excitation: 494	Emission: 520
CAL Fluor® Orange 560	Excitation: 538	Emission: 559
CAL Fluor® Red 610	Excitation: 590	Emission: 610
Cyanine 5	Excitation: 649	Emission: 670

6.4.1 CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

Note: The colour names in the CFX software must not be changed. The PlexTyper® software needs the default names for the interpretation and the correct import:



Channel	Fluorophore	Selected
1	FAM	☑
	SYBR	☐
2	HEX	☑
	TET	☐
	Cal Orange 560	☐
	Cal Gold 540	☐
	VIC	☐
3	ROX	☐
	Texas Red	☑
	Cal Red 610	☐
	Tex 615	☐
4	Cy5	☑
	Quasar 670	☐
5	Quasar 705	☑
	Cy5-5	☐

PCR program:

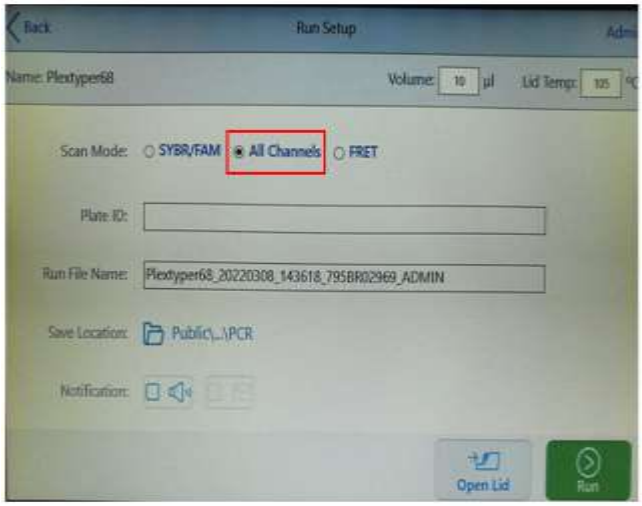
Step	Time [s]	Temperature [°C]	Ramp rate [°C/s]	Plate read	Cycles
Initial activation	120	96	2,5	-	1
Denaturation	5	98	2,5	-	13
Annealing + Extension	25	68	2,2	-	
Denaturation	5	98	2,5	-	37
Annealing + Extension	25	68	*-	Yes	
Cooling	120	37	2,2	-	1

* use the default ramp rate of the CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

Note: Before starting the program choose the correct Scan Mode: All Channels. If the wrong Scan Mode is used the test cannot be interpreted and must be repeated. The lid temperature must be set to 105°C.

CFX96 Touch™

CFX Opus 96



6.4.2 LightCycler® 480 II Real-Time PCR Detection System

Please note that the light source for this cycler has been changed. From serial number 29001 it is an LED lamp, previously it was a xenon lamp. The test was validated on a unit with an LED lamp. It is expected that the older versions will also be compatible with the test, but it is likely that colour compensation will be required. Please contact BAG Diagnostics if you have a device with a xenon lamp and your results are suboptimal.

PCR-Program

According to the operating instructions of the LightCycler® 480 II, create and save a PCR protocol with the following parameters:

Detection Format: FastQ® CD, Block size 96, Reaction volume 10 µl

Step	Cycles	Analysis Mode	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (°C/s)
Hold	1	None	96	None	00:02:00	2.5
Cycle	13	None	98	None	00:00:05	2.5
			68	None	00:00:25	2.2
Cycle	37	Quantification	98	None	00:00:05	2.5
			68	Single	00:00:25	2.2
Hold	1	None	37	None	00:02:00	2.2

Channels for the LightCycler® 480 II Real-Time PCR Detection System

Please use the following channel settings in **Detection Format**:

Excitation	Emission 488	510	580	610	640	660
440						
465		✓				
498						
533			✓	✓		
618						✓

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
465	510	FAM	1	10	1
533	580	O560 (CalFluor Orange560)	1	10	1
533	610	R610 (CalFluor Red610)	1	10	1
618	660	Q670 (Quasar670)	1	10	1

It is strongly recommended to perform the device-specific colour compensation with the RT-CC Universal LC480 Kit (REF 728259) and to use it in PlexTyper® to correct the coefficients. If you have any questions regarding this, please contact BAG Diagnostics customer service at info@bag-diagnostics.com or +49 6404 925125.

Please note the device settings for the plate type: White Plates / Clear Plates

6.4.3 QuantStudio™ 6 Flex System

Experiment properties:

Instrument type:	QuantStudio™ 6 Flex System
Block type:	96-Well (0.2 mL) oder Fast 96-Well (0.1 mL)
Experiment type:	Comparative Ct (ΔΔCt)
Reagent type:	TaqMan® Reagents
Run properties:	Standard

Define Targets:

Target Name	Reporter	Quencher	Color
FAM	FAM	NFQ-MGB	Green
ORANGE560	VIC	NFQ-MGB	Orange
RED610	ROX	NFQ-MGB	Red
Cy5	Cy5	NFQ-MGB	Yellow

Passive Reference: None
Assignment: Assign all targets to each well.
Reaction volume: 10 µl

Run Method:

Stage	Cycles	Data Collection	Target (°C)	Hold (mm:ss)	Ramp rate (°C/s)
Hold Stage	1	Off	96	00:02:00	2.5
PCR Stage	13	Off	98	00:00:05	2.5
			68	00:00:25	2.2
PCR Stage	37	Off	98	00:00:05	2.5
		On	68	00:00:25	2.2
Hold Stage	1	Off	37	00:02:00	2.2

7 INTERPRETATION OF RESULTS WITH THE PlexTyper® SOFTWARE

The evaluation and interpretation of the test results must be carried out with the PlexTyper® software using the validated RT cyclers listed below. Please also refer to the instructions for use for the PlexTyper® software.

- CFX96 Touch™ and CFX Opus 96 Real-Time PCR Detection System, Bio-Rad
- LightCycler® 480 II Real-Time PCR Detection System, Roche Molecular Systems Inc.
- QuantStudio™ 6 Flex System, Applied Biosystems / Thermo Fisher Scientific

Creating tests and worklists in PlexTyper® is described in detail in the instructions for use for the PlexTyper® software.

For software-based evaluation and interpretation of the data, the PlexTyper® software (available free of charge from BAG Diagnostics) is required in conjunction with the PlexTyper® specific kit files. The kit files required for the evaluation are available for download on the download server (www.service.bag-diagnostics.com).

Note the product and lot number of the kit used. The kit files are product and lot specific and also specific for the RT-PCR cycler used. The use of wrong kit files (wrong kit, wrong lot, wrong cycler) can lead to incorrect genotyping.

To evaluate the results, the data must be transferred from the thermal cycler to a computer with the PlexTyper® software (e.g. with a suitable USB stick). Please refer to the PlexTyper® instructions for use for data evaluation.

It is possible, but not necessary, to check the data generally in the thermocycler software. For example, valid tests must show sufficient fluorescence signals in the FAM channel of the internal control. Positive reactions show a positive colour signal in the corresponding colour channel.

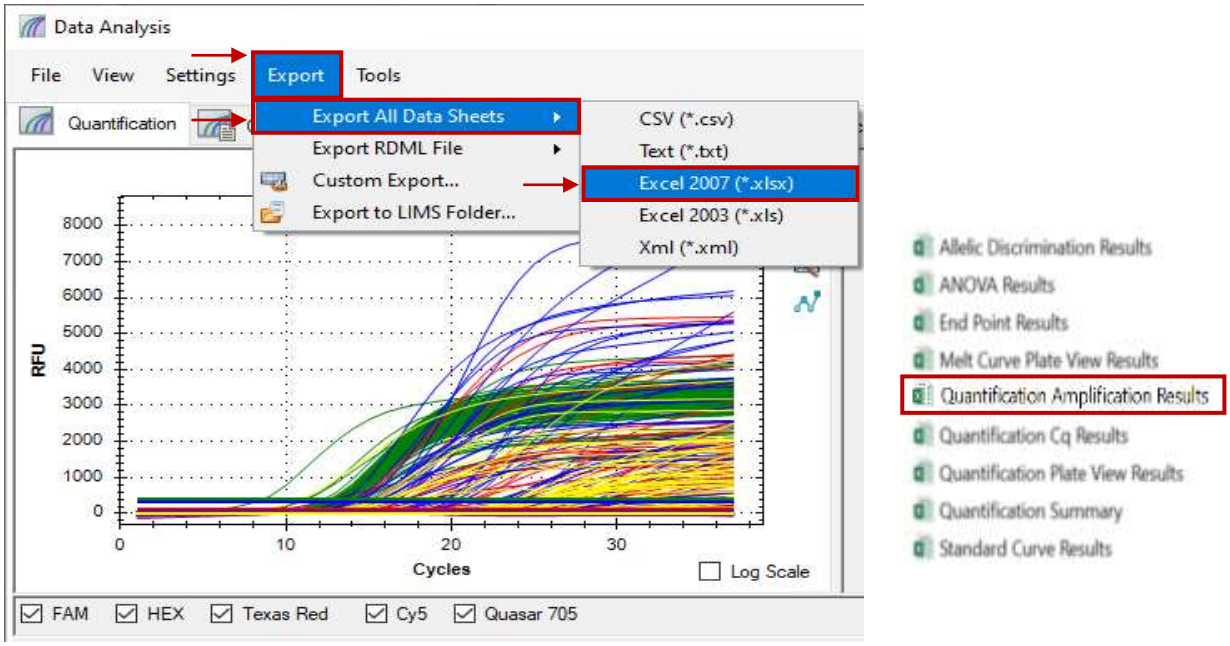
A negative control (NTC) serves as a contamination control. If DNA or contaminating amplicons are unintentionally added to the NTC reaction, this leads to a positive signal. If the Cq is below 36, this indicates possible contamination. Amplification signals with a higher Cq than 36 in the NTC are considered PCR artefacts and are not taken into account. If PCR contamination is suspected, it is recommended to decontaminate the PCR laboratory from DNA and to exchange the reagents.

The raw data determined by the cycler-specific software are imported into the PlexTyper® software. For this, an export of the cycler-specific raw data must be carried out in advance, as described in section 7.1. The PlexTyper® software uses the Cq values, RFUs (Relative Fluorescence Units) and the shape of the amplification curve to determine the positive and negative reactions from which the molecular genetic HLA-DQ characteristics of the samples used are determined.

7.1 Export of results from the cycler

7.1.1 CFX96 Touch™ & CFX Opus 96 Real-Time PCR Detection System

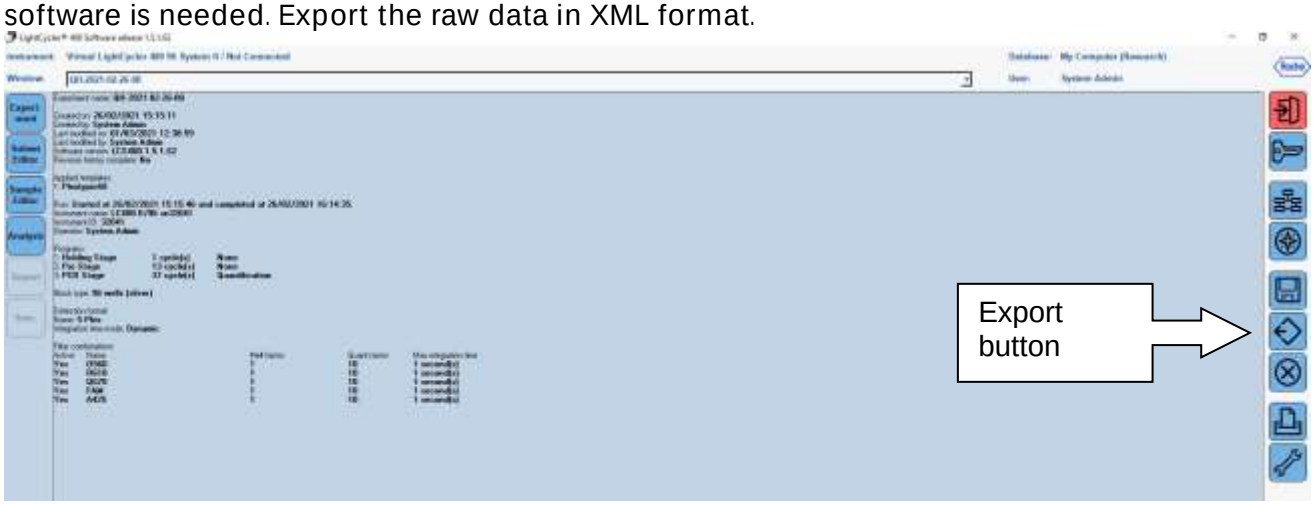
Open the data file with the CFX software and export the Excel 2007 file (.xlsx).



Note: **Only the file "Quantification Amplification Results" is needed. It makes sense to delete the other files.**

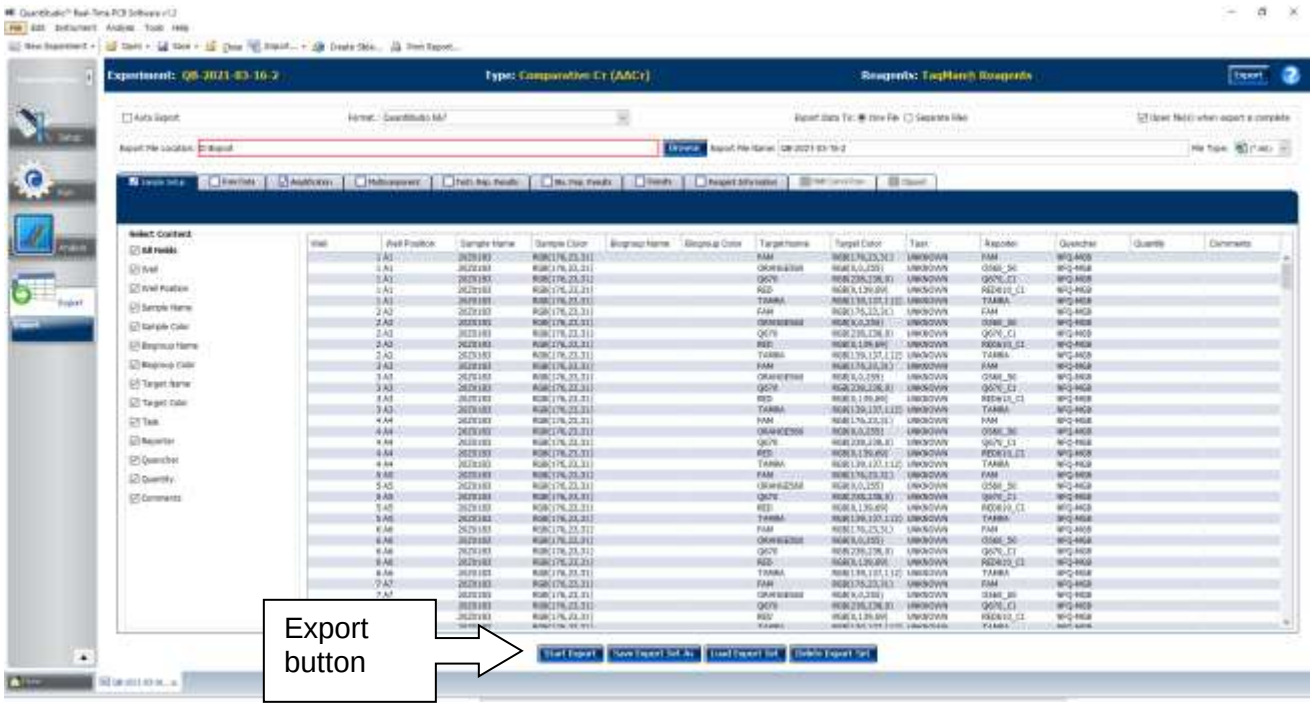
7.1.2 LightCycler® 480 II Real-Time PCR Detection System

PlexTyper® uses xml files from the LightCycler® 480 II. After the run no analysis in the Roche software is needed. Export the raw data in XML format.



7.1.3 QuantStudio™ 6 Flex System

Open the Export menu and start the export of the "Sample Setup" and the "Amplification" tab as (*.xls) file.



7.2 Evaluation and interpretation

The PlexTyper® software receives raw data from the amplification files of the supported real-time devices and calculates the data for the Cq value. It also analyses the quality of the amplification and automatically assigns positive and negative reactions based on this.

The PlexTyper® kit files contain the threshold values for the reactions and the HLA-DQ specificities for each reaction in each colour channel. The possible HLA-DQ genotypes are calculated from the pattern of positive and negative reactions. The possible genotypes are displayed to the user and the user can accept or edit the genotype.

The HLA-DQ genotypes are grouped into disease associated allele strings (see below) and the allele filters do not have an effect for this kit (common and well documented alleles =CWD (Mack et al 2013)).

7.2.1 Result histogram

The result histogram shows all reactions for a test. The colour of the bars indicates the colour channel in which the reaction is detected. Positive reactions are directed upwards, negative ones downwards. The higher the bar, the more clearly positive or negative a reaction is rated. A detailed description of the QS (QS = Quality Score) values can be found in the instructions for use for the PlexTyper® software.

The green field above the histogram represents the internal amplification control. If this fails, the field turns white and contains a "-". The 

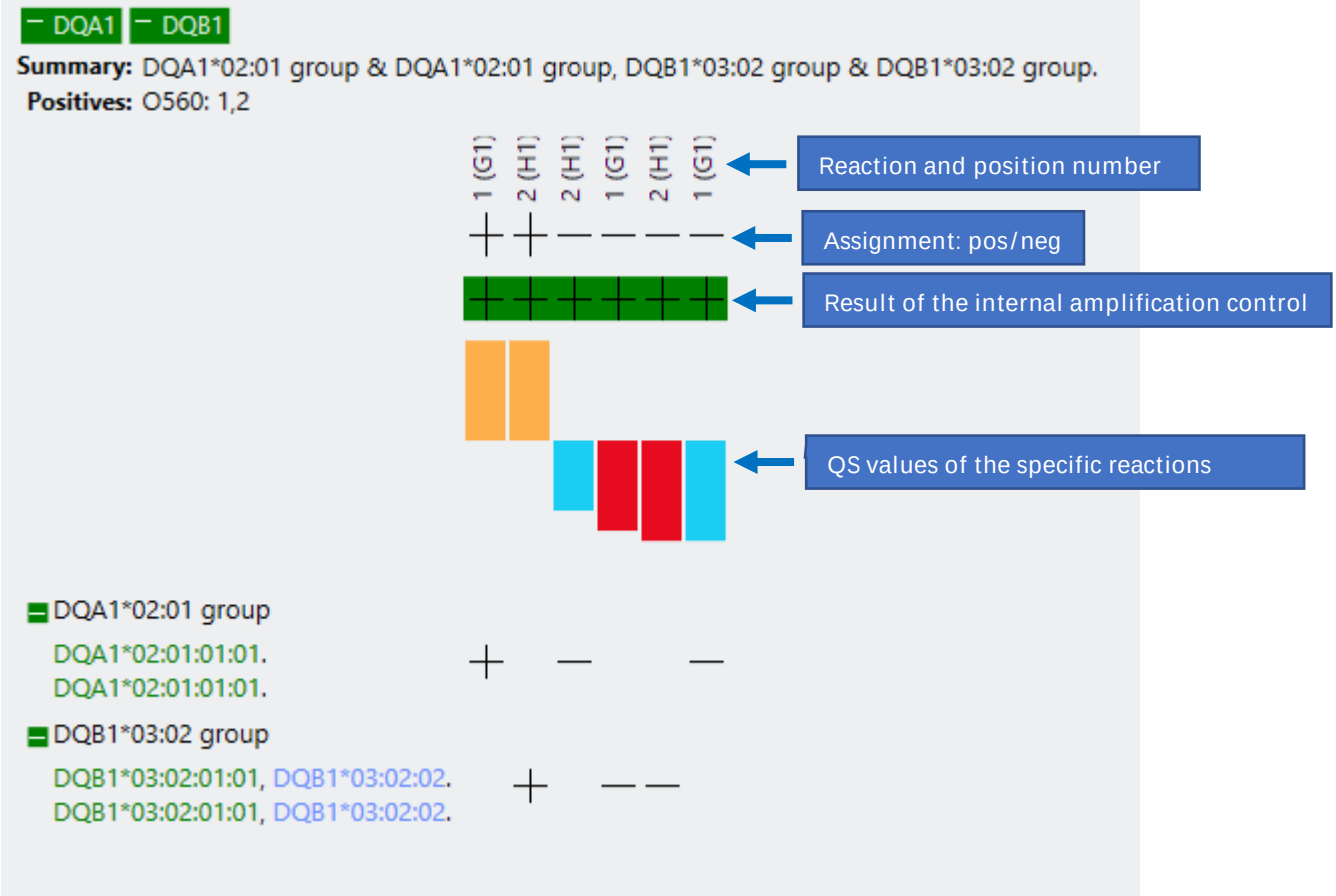
buttons in the upper right corner can be used to enlarge or reduce the size of the histogram.

The FastQ® CD kit focuses on the differentiation between the coeliac disease associated alleles DQA1*02:01, DQA1*05:01, DQA1*05:05 and DQB1*02:01, DQB1*02:02, DQB1*03:02 from other non-associated alleles. Therefore, no full HLA-DQ genotype is generated.

The default view of the results histogram does only show the DQA1 locus. Pressing the grey DQB1 button this locus is displayed as well: 

By default, the results are summarised in the histogram. In case of positive results, the button  expands the results and shows the allele group strings with the respective reaction patterns.

Above the histogram is a summary of the result, e.g. DQA1*02:01 group & DQA1*02:01 group and a mention of the positive reactions ("Positives"). The representation as DQA1*02:01 group & DQA1*02:01 group comes from the evaluation of complete HLA typings in the same software and is not entirely correct, as it can be a homozygous or a heterozygous result for DQA1.



7.2.2 Coeliac disease associated allele groups

The reaction mixes detect common and well documented alleles as well as rare alleles (see 7.5 Kit Specificity). To simplifiy the display CWD groups were defined which basically contain only the CWD alleles:

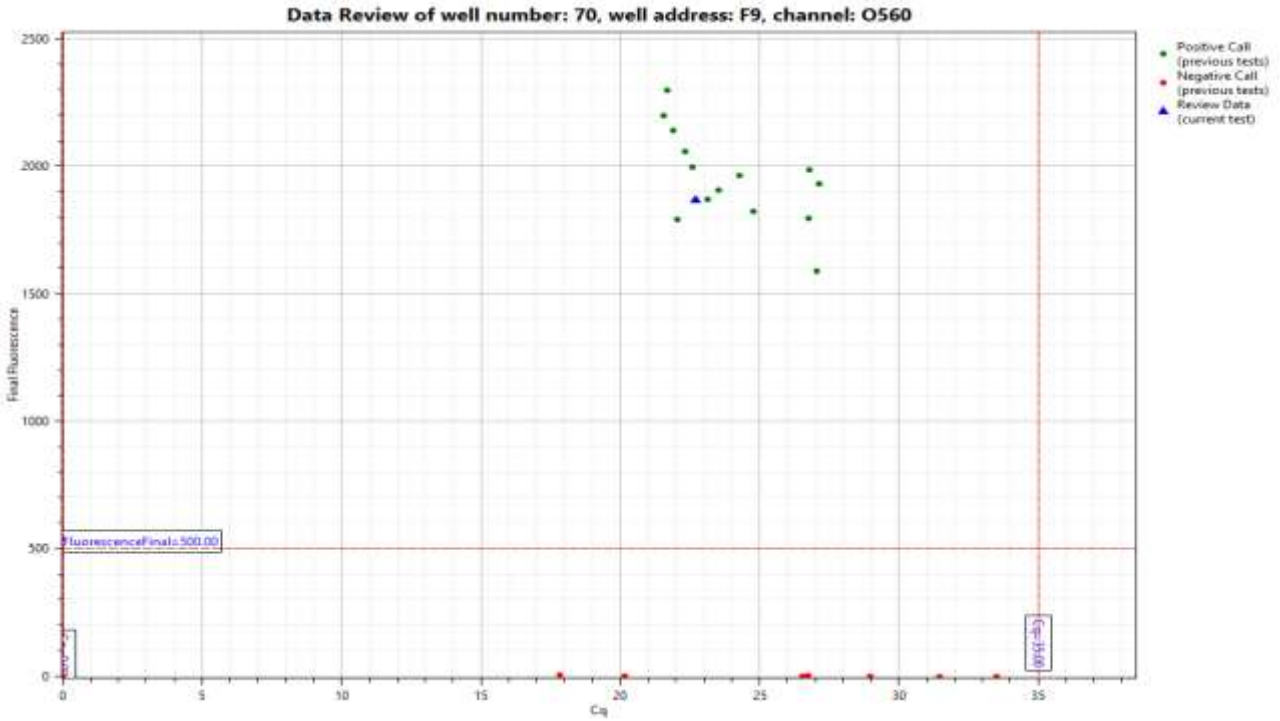
- DQA1*05:05/08/09 group:
  DQA1*05:05:01:01 (C)
  DQA1*05:08 (C)
  DQA1*05:09:01:01 (WD)
  not included because of unknown sequence in exon 3: DQA1*05:02 (WD)
- DQA1*05:01/03 group:
  DQA1*05:01:01:01 (C)
  DQA1*05:03:01:01 (C)
  not included because of unknown sequence in exon 3: DQA1*05:02 (WD)
- DQA1*02:01 group:
  DQA1*02:01:01:01 (C)
- DQB1*02:01 group:
  DQB1*02:01:01:01 (C)
- DQB1*02:02 group:
  DQB1*02:02:01:01 (C)
  DQB1*02:180 (C) – formerly known as DQB1*02:03

- DQB1*03:02 group:
  DQB1*03:02:01:01 (C)
  DQB1*03:02:02 (WD)

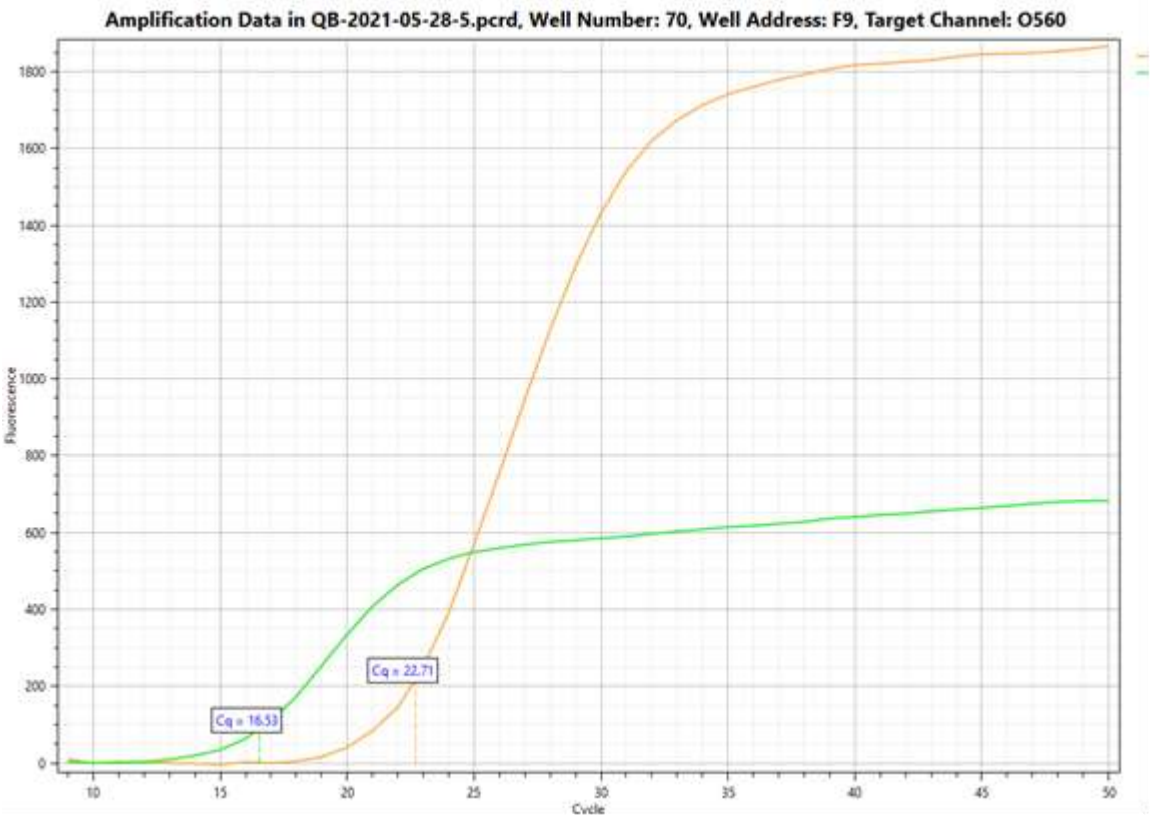
7.2.3 Interpretation tools

There are some tools available in PlexTyper® that can be useful when the automatic interpretation does not find a result or if there is a rare result that should be checked. Most of these tools are intended for a complete HLA typing and are not useful for the evaluation of the FastQ® CD kit. A detailed description can be found in the Instructions for Use for the PlexTyper® software. In general, reactions with a poor quality score (QS value between +3 and -3) should be checked.

Double-clicking on bars for the QS value opens a diagram showing the Cq value and the final fluorescence of the reaction in the context of other reactions with the same kit lot:

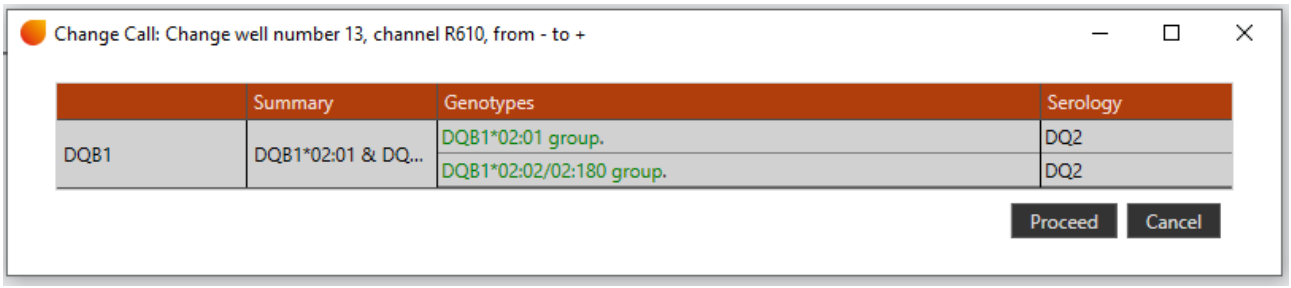


The red lines indicate the thresholds for positive reactions. The blue triangle represents the currently selected test. Double-clicking on the blue triangle opens a window with the amplification curves for the internal amplification control (green) and the HLA-DQ specific reaction (orange). In case of a poor QS value, check whether the reaction is close to one of the threshold values and whether the amplification curve has a sigmoidal shape.

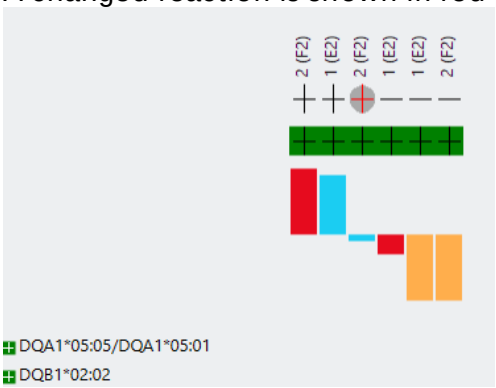


7.2.4 Change a reaction call

If the software has incorrectly assessed a reaction with a poor QS value, this assessment can be changed manually. All changes by the user are logged and displayed in the audit trail in the results report. With a right mouse click on the corresponding bar in the histogram, a preview of the effect of a change in the reaction can be opened (Preview effect of change from + to - or in the other direction). Then select either Proceed to change the result or Cancel to discard the change.

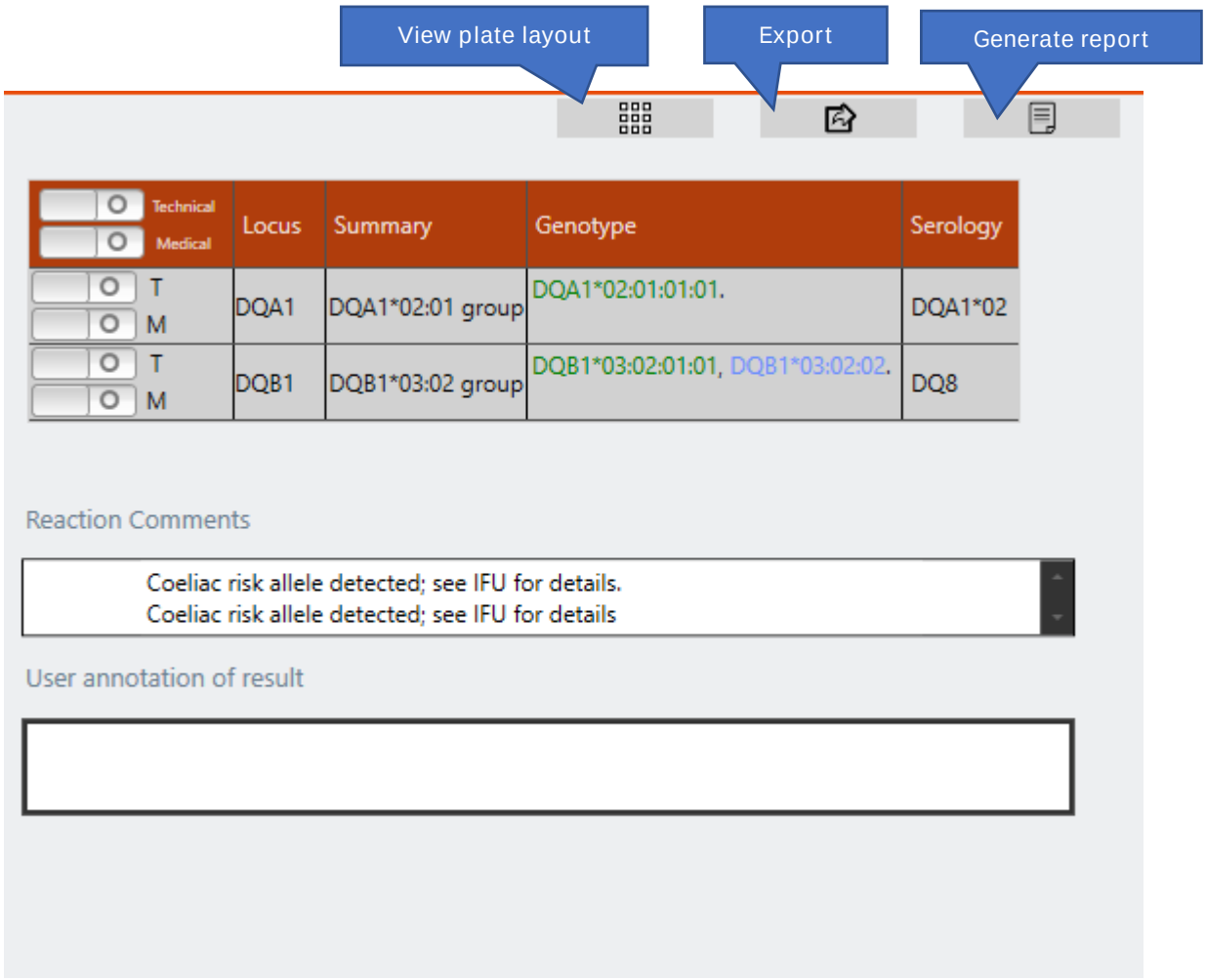


A changed reaction is shown in red in the histogram as shown in the figure.



7.3 Results view

On the right side of the screen, the results are displayed in a table containing the following information: Locus, results summary, genotype as DQ group, serological equivalent and the approval status. From this table, the results can be exported to a text file using the Export button and a PDF report can be generated using the Create Report button. The View Plate button displays the plate layout as an image that can be copied to the clipboard..



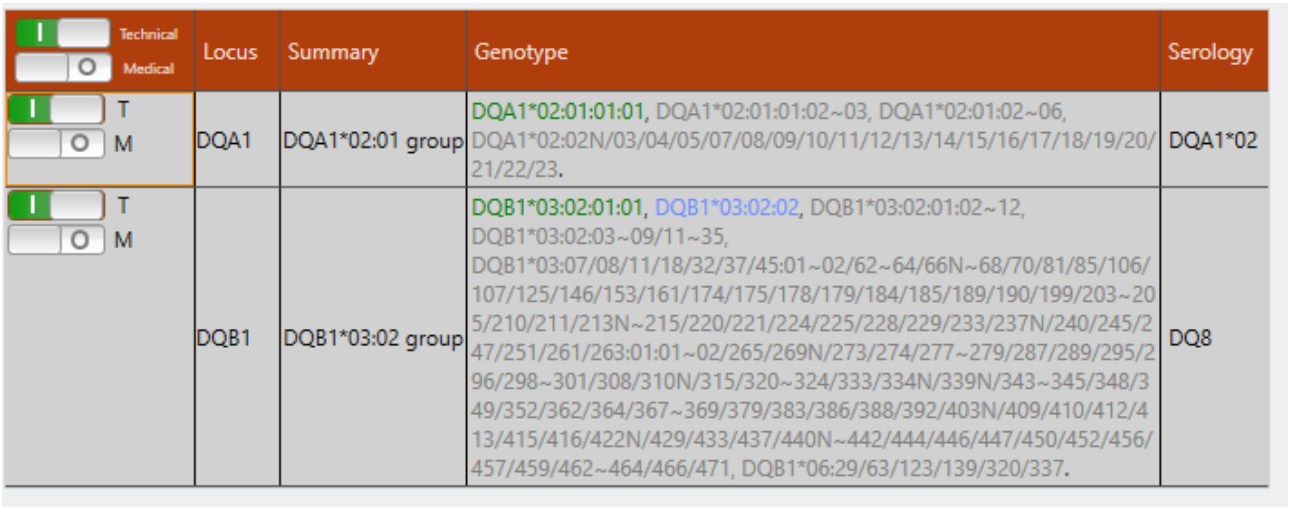
In the Reaction Comments field, comments generated by the software regarding the presence of coeliac disease associated alleles are displayed. If none of the listed associated alleles is detetced the summary states "DQA1* No DQA Coeliac alleles identified" or "DQB1* No DQB Coeliac alleles identified"

In another field at the bottom of the window, comments can be entered by the user (User annotation of result).

A two-step approval process is implemented in the software. The Technical approval -T can be done either by a user with the role Technician or Supervisor. To do this, the button in the first column is pressed and it then turns green. In a second step, the Medical approval (-M) is carried out exclusively by a Supervisor. The approval can be done for all gene loci together in the header line or individually in the respective lines with the gene loci.

If a gene locus has been approved (either technically or medically) and a reaction score is subsequently changed for this result, the approvals are all removed (initially a warning is displayed).

If All is selected as allele filter, rare alleles are displayed in the Genotype section as well:



Technical / Medical	Locus	Summary	Genotype	Serology
T / M	DQA1	DQA1*02:01 group	DQA1*02:01:01:01, DQA1*02:01:01:02~03, DQA1*02:01:02~06, DQA1*02:02N/03/04/05/07/08/09/10/11/12/13/14/15/16/17/18/19/20/21/22/23.	DQA1*02
T / M	DQB1	DQB1*03:02 group	DQB1*03:02:01:01, DQB1*03:02:02, DQB1*03:02:01:02~12, DQB1*03:02:03~09/11~35, DQB1*03:07/08/11/18/32/37/45:01~02/62~64/66N~68/70/81/85/106/107/125/146/153/161/174/175/178/179/184/185/189/190/199/203~205/210/211/213N~215/220/221/224/225/228/229/233/237N/240/245/247/251/261/263:01:01~02/265/269N/273/274/277~279/287/289/295/296/298~301/308/310N/315/320~324/333/334N/339N/343~345/348/349/352/362/364/367~369/379/383/386/388/392/403N/409/410/412/413/415/416/422N/429/433/437/440N~442/444/446/447/450/452/456/457/459/462~464/466/471, DQB1*06:29/63/123/139/320/337.	DQ8

In the header above the result histogram, information on the sample, the kit used and the cycler used is given.

7.4 Coeliac disease risk assignment

The table below indicates the risk for coeliac disease associated with expected outcomes of the FastQ® CD kit. The risk assignment is based on the data presented in Megiorni and Pizzuti (2012) and Sollid and Lie (2005).

1	DQB1*02:01	DQA1*05:05	Highest risk
	DQB1*03:02	-	
2	DQB1*02:01	DQA1*05:05	High risk
3	DQB1*02:01	DQA1*05:05	High risk
	-	DQA1*05:01	
4	DQB1*02:01	DQA1*05:05	High Risk
	DQB1*02:02	DQA1*02:01	
5	DQB1*02:02	DQA1*02:01	High Risk
	DQB1*03:02	-	

6	DQB1*03:02	-	High Risk
7	DQB1*03:02	DQA1*05:01	High Risk
8	DQB1*03:02	DQA1*05:05	High Risk
9	DQB1*02:02	DQA1*05:01	Low Risk
10	DQB1*02:02	DQA1*05:05	Low Risk
11	DQB1*02:02	DQA1*02:01	Low Risk
12	DQB1*02:02	-	Low Risk
13		DQA1*05:01	Very low risk
		DQA1*05:05	
14		DQA1*05:01	Very low risk
15		DQA1*05:05	Very low risk
16	-	-	Coeliac disease unlikely

7.5 Specificity of the kit

The following alleles are detected by the kit:

Primermix I	Common & well documented*	Rare*
CAL Fluor® Red 610	**DQB1*02:01:01:01**	*DQB1*02:01:01:02~13, *02:01:04~08, *02:01:24~42, *02:03:02, *02:07:01,*02:08, *02:09, *02:14:01:01~02, *02:27, *02:48, *02:53Q, *02:57, *02:59, *02:63, *02:72, *02:79, *02:81, *02:82, *02:83, *02:93, *02:96N, *02:98, *02:99, *02:102, *02:105~109, *02:111, *02:112, *02:114, *02:115, *02:118, *02:119, *02:123, *02:125, *02:128, *02:130, *02:132N, *02:133, *02:134N, *02:135, *02:136, *02:148, *02:149, *02:152, *02:154, *02:155, *02:157, *02:158~160, *02:163N, *02:164, *02:166, *02:170, *02:174, *02:178, *02:182, *02:184~186, *02:188~193, *02:196~198.*
Cyanine 5	**DQA1*05:05:01:01** **DQA1*05:08** **DQA1*05:09** **DQA1*05:02**	*DQA1*05:05:01:02~30, *05:05:02 ~10, *05:09:01:02, *05:10~14, *05:17N, *05:20, *05:21, *05:24~26, *05:28, *05:29Q, *05:39, *05:42~46, *05:48, *05:50, *05:51.*
CAL Fluor® Orange 560	**DQA1*02:01:01:01**	*DQA1*02:01:01:02~*02:23.*

Primermix II	Common & well documented*	Rare*
CAL Fluor® Red 610	**DQB1*02:02:01:01, DQB1*02:180.**	*DQB1*02:02:01:02~12, *02:02:02, *02:02:06:02, *02:02:07~17, *02:06, *02:10, *02:11, *02:12, *02:20N, *02:26, *02:50, *02:62, *02:64, *02:65, *02:71, *02:80, *02:84, *02:89:01, *02:89:02, *02:95, *02:97, *02:110, *02:113, *02:116, *02:117, *02:120~122, *02:124, *02:126, *02:127, *02:131, *02:137, *02:138, *02:141, *02:142:01:01~147, *02:150, *02:153, *02:156, *02:161, *02:162N, *02:165, *02:167N, *02:169, *02:171Q, *02:172, *02:175, *02:176N, *02:179, *02:183N, *02:187, *02:194N, *02:195, DQB1*03:01:01:26.*
Cyanine 5	**DQA1*05:01:01:01, DQA1*05:03:01:01**	*DQA1*05:01:01:02~07, *05:03:01:02, *05:03:02, *05:06:01:01~03, *05:07, *05:15N, *05:18, *05:19, *05:22, *05:23, *05:27, *05:31, *05:33, *05:35, *05:36N, *05:38, *05:40, *05:41, *05:47, *05:49.*
CAL Fluor® Orange 560	**DQB1*03:02:01:01 DQB1*03:02:02.**	*DQB1*03:02:01:02~12, *03:02:03~09, *03:02:11~35, *03:07, *03:08, *03:11, *03:18, *03:32, *03:37, *03:45:01, *03:45:02, *03:62~68, *03:70, *03:81, *03:85, *03:106, *03:107, *03:125, *03:146, *03:153, *03:161, *03:174, *03:175, *03:178, *03:179, *03:184, *03:185, *03:189, *03:190, *03:199, *03:203~205, *03:210, *03:211, *03:213N, *03:214, *03:215, *03:220, *03:221, *03:224, *03:225, *03:228, *03:229, *03:233, *03:237N, *03:240, *03:245, *03:247, *03:251, *03:261, *03:263:01:01, *03:263:01:02, *03:265, *03:269N, *03:273, *03:274, *03:277, *03:278, *03:279, *03:287, *03:289, *03:295, *03:296, *03:298~301, *03:308, *03:310N, *03:315, *03:320~324, *03:333, *03:334N, *03:339N, *03:343~345, *03:348, *03:349, *03:352, *03:362, *03:364, *03:367~369, *03:379, *03:383, *03:386, *03:388, *03:392, *03:403N, *03:409, *03:410, *03:412, *03:413, *03:415, *03:416, *03:422N, *03:429, *03:433, *03:437, *03:440N, *03:441, *03:442, *03:444, *03:446, *03:447, *03:450, *03:452, *03:456, *03:457, *03:459, *03:462~464, *03:466, *03:471, DQB1*06:29, *06:63, *06:123, *06:139, *06:320, *06:337.*
Rare alleles of unknown reactivity due to incomplete sequences		
DQA1*05 alleles that are not completely sequenced in exon 3 so may be amplified in either reaction one, or reaction two, red 610 channel.		*DQA1*05:01:02, *05:02 (DQA1*05:02 is listed as CWD), *05:04, *05:16, *05:30, *05:32, *05:34, *05:37.*
DQB1*02 alleles that are not completely sequenced in exon 3 so may be amplified in either reaction one, or reaction two, red 610 channel.		*DQB1*02:01:02~03, *02:01:09, *02:01:11 ~13, *02:01:15~23, *02:05, *02:07:02, *02:13, *02:14:02, *02:15~*02:19, *02:21~25, *02:28~*47, *02:49, *02:51, *02:52, *02:54~56, *02:58N, *02:60, *02:61, *02:66~ *02:70, *02:73~78, *02:85~88, *02:90, *02:91, *02:92, *02:94, *02:100, *02:101, *02:103, *02:104, *02:129N, *02:139, *02:140, *02:151, *02:168, *02:173, *02:177N, *02:181.*
DQB1*02 alleles not detected in this kit		*DQB1*02:04.*

IMGT Database 3.47.0

* Common and well documented alleles based on CWD 2.0.0 catalogue (Mack et al 2013) with modifications for later sequence updates

8 WARNINGS AND PRECAUTIONS

The **FastQ® CD kit** is designed for in vitro diagnostic use and should be used by properly trained, qualified staff only. All work should be performed using Good Laboratory Practices.

Biological material used for extraction of DNA, e.g. blood, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.

The Plex Mix contains the hazardous substance 2-methylisothiazol-3(2H)-one at a concentration of < 0.05%. The following hazardous material labeling is applicable:

Symbol: Warning

See chapter 14 for hazard and precaution statements.

A material safety data sheet (MSDS) for the Plex Mix is available for download from www.bag-diagnostics.com. No further MSDS are required according to article 31 of REACH regulation (EC) no. 1907/2006 and the regulation (EC) no. 1272/2008.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

For the FastQ® CD kit a performance evaluation study with pre-typed DNA samples were performed. The results from the study were compared to the results that were obtained with other CE certified typing reagents (amongst others serology, SSO, SSP) and/or sequencing. No discrepancies in the detection of the HLA DQ feature have been observed (100 % concordance).

Q Primermix I

Genotypes	No. of samples CFX	No. of samples LC 480 II	No. of samples QS 6	Concordance with pre-typing
DQB1*02:01 positive	11	11	11	100%
DQA1*05:05, DQA1*05:08, DQA1*05:09, DQA1*05:02 positive	28	28	28	100%
DQA1*02:01 positive	22	22	22	100%
DQB1*02:01, DQA1*05:05, DQA1*05:08, DQA1*05:09, DQA1*05:02, DQA1*02:01 negative	200	200	200	100%
Total	**261**	**261**	**261**	**100%**

Q Primermix II

Genotypes	No. of samples CFX	No. of samples LC 480 II	No. of samples QS 6	Concordance with pre-typing
DQB1*02:02, DQB1*02:180 positve	21	21	21	100
DQA1*05:01, DQA1*05:03 positive	14	14	14	100
DQB1*03:02 positive	12	12	12	100
DQB1*02:02, DQB1*02:180, DQA1*05:01, DQA1*05:03, DQB1*03:02 negative	214	214	214	100
Total	**261**	**261**	**261**	**100**

10 LIMITATIONS OF THE METHOD

Because of the high susceptibility of the RT-PCR method for cross contaminations special care should be taken during DNA isolation. Validation tests in the course of the performance evaluation study of the **FastQ® CD** kit have shown that a variation of the amount of DNA used for the amplification between 5 ng and 50 ng do not have a significant influence on the detection of the HLA-DQ alleles.

Extreme care should be taken to prevent contamination of the kit reagents and other laboratory materials and equipment with amplicons or DNA. Regular wipe tests (e.g. BAG Wipe Test, REF 7091) and negative controls with Aqua dest with each assay are strongly recommended.

In the negative control with Aqua dest. there must not be any fluorescent signal (Cq > N.A.). In the case of signal development in the negative control the PCR working place has to be decontaminated and the reagents have to be exchanged if necessary. All instruments (e.g. pipettes, realtime cyclers) must be calibrated according to the manufacturers instructions.

11 INTERNAL QUALITY CONTROL

Internal quality control of new lots of the **FastQ® CD** kit can be performed using a combination of DNA specimens with known HLA type. An internal positive control for successful amplification is contained in the Q Primermixes I and II. Negative controls to detect possible contaminations are recommended. Use a PCR reaction without DNA (NTC) for this purpose.

12 TROUBLESHOOTING

Symptom	Possible reason	Potential solution
Poor or no signal	Presence of an inhibitor in the PCR-reaction	Use fresh reagents
	Insufficient amount of DNA in the reaction	Repeat test with correct amount of DNA
	Wrong amplification parameters	Check PCR program
	Contaminated or degraded DNA	Check concentration and quality of the DNA Check DNA on a gel Repeat DNA isolation
	Degraded fluorescent probes or primers	Use fresh Q Primermix Avoid exposure to light and frequent thawing and freezing Pay attention to storage conditions
	Bubbles in the PCR reaction, residual liquid at the inner wall of the tube	Careful pipetting Spin down PCR plate
	Incompatible or low quality RT-PCR plastics	Use compatible and high quality plastics
	Wrong signal calculation due to abnormal amplification signals during the initial cycles of the run	Application of corrective measures in the software (e.g. "apply fluorescence drift correction" function from Bio-Rad or exclusion of the first five cycles from analysis)
	Evaporation of the reagents due to incorrect closing of the PCR tubes	Make sure that the PCR tubes are closed properly Caution with adhesive foils in the edge areas
Signal in the negative control	Contamination with DNA in the negative control	Repeat the test - decontaminate the workplace

13 TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

TaqMan® is a trademark of Roche Molecular Systems Inc.

®Cal Fluor & Quasar Dyes are registered trademarks of LGC Biosearch Technologies

LightCycler® is a registered trade mark by the company Roche Molecular Systems Inc.

QuantStudio™ is a registered trade mark by the company Applied Biosystems / Thermo Fisher Scientific.

FrameStar® is a registered trade mark by the company Azenta Life Sciences

14 EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Upper limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
CONT	Content, contains
GENOTYPING	Intended use: Typing of human genetic markers that are associated with diseases or pharmacogenetic reactions
eIFU	Electronic instructions for use
IVD	For in vitro diagnostic use
LOT	Batch code
PLEX MIX	Mastermix for RT-PCR
Q Primermix | CD I	Primermix number I for the detection of HLA-DQ-attributes with the FastQ® CD kit
Q Primermix | CD II	Primermix number II for the detection of HLA-DQ-attributes with the FastQ® CD kit
REF	Catalogue number
	Warning (see chapter 8) H317 May cause an allergic skin reaction. **Precautionary statements** P101 If medical advice is needed, have product container or label at hand. P102 Keep out of reach of children. P103 Read carefully and follow all instructions. P302+P352 IF ON SKIN: Wash with plenty of water. P280 Wear protective gloves / protective clothing / eye protection / face protection / hearing protection. P333+P313 If skin irritation or rash occurs: Get medical advice/ attention. P501 Dispose of contents/container in accordance with local/ national regulation.

15 LITERATURE

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