CeliaSCAN

Assay for the rapid detection of HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8

CE Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices

IVD **For In Vitro Diagnostic Use**

Test Instructions

Multiplex Real-Time PCR Assay and Melting Curve Analysis for the *in vitro* detection of HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8

Store at **-20ºC** upon receipt

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1. Intended use

The CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay kit is intended to be used for *in vitro* detection of the MHC class II HLA-DQ2.5 (hetero- and homozygous), HLA-DQ8, HLA-DQ2.2 and *HLA-DQA1*05* markers in human DNA.
The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. No special training will be required for professional routine diagnostic laboratories performing quantitative polymerase chain reaction (PCR).

2. Summary and explanation of the test

Celiac disease (CD) is an autoimmune disease characterized by chronic diarrhoea, inflammatory lesions in the small bowel, villous atrophy, nutritional malabsorption and occasionally neurological symptoms. Once thought to be a rare disease, CD is increasingly being diagnosed and currently has a worldwide prevalence of 1%. Gluten, the primary component of wheat, rye and barley, is the environmental agent responsible for CD. Ninety per cent of CD patients express the human leukocyte antigen (*HLA*)-*DQA1*05*, *HLA-DQB1*02* heterodimer (HLA-DQ2.5). Of the remaining 10% most carry the *HLA-DQA1*03*, *HLA-DQB1*03:02* heterodimer (HLA-DQ8). A small minority of CD patients carries only *HLA-DQA1*05* or *HLA-DQB1*02* (usually on the *HLA-DQA1*02 - HLA-DQB1*02* haplotype (HLA-DQ2.2). Individuals with the highest risk for developing CD are those homozygous for *HLA-DQA1*05 - HLA-DQB1*02 (*HLA-DQ2.5 homozygotes).
CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay kit is an easy and ready to use real-time 96-well PCR assay and melting curve analysis that combines speed and accuracy. It provides 100% sensitivity and specificity.

3. Principle of the test procedure

The detection of HLA-DQ2.5, HLA-DQ2.2, HLA-DQ8 and *HLA-DQA1*05* markers by PCR is based on the amplification of allele specific parts in exon 2 of both the *HLA-DQA1* and *HLA-DQB1* genes. Six primer pairs detect the DQA1*02, DQA1*03, DQA1*05, DQB1*02, DQB1*0302 and DQ2.5 homozygous SNP alleles needed to successfully genotype for DQ2 and DQ8. Two more additional primer pairs detect monomorphic parts of the HLA-DRA and HLA-F genes and serve as amplification and temperature shift controls.
In the real-time PCR melting analysis method, amplification of these specifically selected sequences of DNA is detected by measurement of the SYBR® GreenER™ fluorescence signal. Because SYBR Green is incorporated into all double-stranded DNA, a melting curve must be performed to determine if the correct alleles have been amplified. The melting curve is performed after the completion of PCR by slowly heating the reaction to 97ºC, which causes melting of the double-stranded DNA and a corresponding sharp decrease of SYBR® GreenER™ fluorescence is seen. The instrument continuously monitors this fluorescence decrease and displays it as melting peaks. Each melting peak represents the characteristic melting temperature,
*T*m, of a particular DNA product (where the DNA is 50% double-stranded and 50% single-stranded). The *T*m is determined at the inflection point of the melting curve. Thus the presence of the desired-specific PCR product in a given sample will be indicated by its primer-specific *T*m.

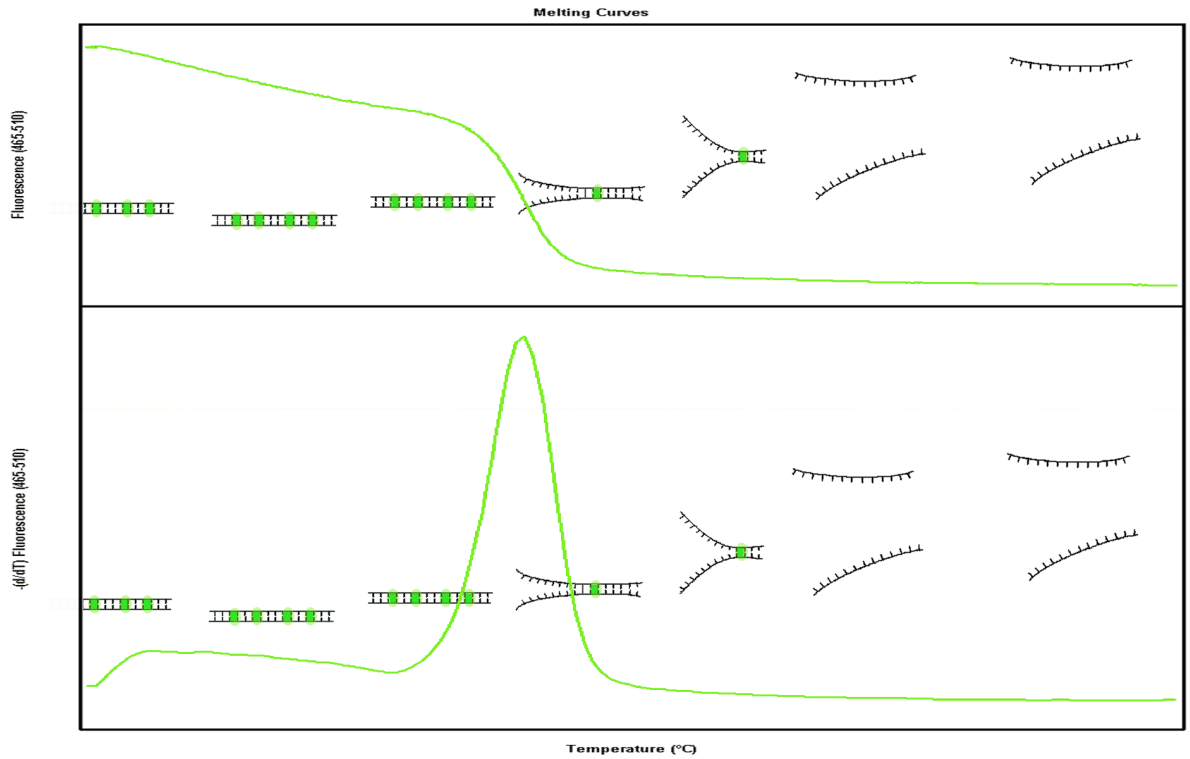


Figure 1: Melting curve analysis principle

4. Reagents

PRECAUTIONS

CAUTION: Handle patient samples as Biohazardous material.
Handle samples as if capable of transmitting an infectious agent.

All clinical samples should be regarded as infectious. These samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious specimen in the Centre for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

Wear protective clothes

Use sterile aerosol resistant pipette tips

A uni-directional workflow must be adhered to in the laboratory with different rooms for sample preparation, pre-amplification area and post-amplification.

4.1 Components in each CeliaSCAN assay kit

1. MasterMix1
   3 tubes with a black colour code, labelled “MasterMix1” each containing 200 µl ready to use PCR mix. This mixture contains eight primers for amplification of three HLA-DQ alleles and HLA-F control. It contains the SYBR® GreenER™ intercalating dye for detection of double stranded DNA. The master mix contains all ingredients for PCR amplification including Taq polymerase.

   MasterMix1

2. MasterMix2
   3 tubes with a blue colour code, labelled “MasterMix2” each containing 200 µl ready to use PCR mix. This mixture contains six primers for amplification of two HLA-DQ alleles and HLA-DRA control. It contains the SYBR® GreenER™ intercalating dye for detection of double stranded DNA. The master mix contains all ingredients for PCR amplification including Taq polymerase.

   MasterMix2

3. MasterMix3
   3 tubes with a green colour code, labelled “MasterMix3” each containing 200 µl ready to use PCR mix. This mixture contains four primers for amplification of one HLA-DQ allele and HLA-DRA control. It contains the SYBR® GreenER™ intercalating dye for detection of double stranded DNA. The master mix contains all ingredients for PCR amplification including Taq polymerase.

   MasterMix3

4. Negative Control
   4 tubes with a transparent colour code, labelled ”Negative Control” each containing 200 µl negative template control.

   Negative Control

5. Positive Control MM1
   4 tubes with a red colour code, labelled ”Positive Control MM1” each containing 20 µl positive control for MasterMix1.

   Positive Control MM1

6. Positive Control MM2
   4 tubes with a yellow colour code, labelled ”Positive Control MM2” each containing 20 µl positive control for MasterMix2.

   Positive Control MM2

7. Positive Control MM3
   4 tubes with a purple colour code, labelled ”Positive Control MM3” each containing 20 µl positive control for MasterMix3.

   Positive Control MM3

Note: Use all components of the same kit lot number.

4.2 Additional required equipment

PCR amplification can be carried out on the Roche LightCycler® 480, the Applied Biosystems® 7500 Real-Time PCR Systems, the Applied Biosystems StepOne and StepOnePlus Real-Time PCR Systems and the BioRad CFX96 real-time PCR detection system.

Use sterile DNase-free polypropylene disposables for all steps in the procedure.

4.3 Specimen collection and preparation of DNA samples

The CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay kit is intended to be used on genomic DNA extracted from whole blood, leucocyte pellets and buccal swab specimens. Standard DNA isolation procedures can be used according to the manufacturer's protocols.

DNA samples must be diluted to a concentration of 20 ng/µl.

4.4 Reagent preparation and storage

For the following 7 components thaw the tube prior to opening. Opened vial can be refrozen and thawed again up to five times. Remark: vortex mix and spin down in a 3 seconds centrifugation step before use: Make note of the number of times thaw/freeze cycles.

1. **Master Mix1**
2. **Master Mix2**
3. **Master Mix3**
4. **Negative Control.**
5. **Positive Control MM1.**
6. **Positive Control MM2.**
7. **Positive Control MM3.**

Store all components at or below -20°C or at 2°C–8°C when the kit will be used again within 7 days.
All components are temperature sensitive. Thaw only the components that are going to be used. Components can be refrozen up to five times. Store the components at 2°C–8°C for no longer than 7 days.

4.5 Chemical or physical indications of instability

Alteration in the physical appearance of test kit materials may indicate instability or deterioration. Expiry dates shown on component labels indicate the date beyond which components should not be used.

5. Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use aerosol resistant pipette tips during the whole test procedure.

5.1 Reagent Preparation

Thaw the mixes needed.

5.2 CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay kit test procedure

1. In the 96-well format prepare reactions in the required number of wells for the number of specimens to be measured, plus one well for the positive control (PC) and one well for the negative template control (NC).

2. Add 15 µl of mastermix1, mastermix2 and mastermix3 to each reaction according to the scheme below.

	MasterMix1				MasterMix2				MasterMix3			
	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample1	Sample9	Sample17	Sample25	Sample1	Sample9	Sample17	Sample25	Sample1	Sample9	Sample17	Sample25
B	Sample2	Sample10	Sample18	Sample26	Sample2	Sample10	Sample18	Sample26	Sample2	Sample10	Sample18	Sample26
C	Sample3	Sample11	Sample19	Sample27	Sample3	Sample11	Sample19	Sample27	Sample3	Sample11	Sample19	Sample27
D	Sample4	Sample12	Sample20	Sample28	Sample4	Sample12	Sample20	Sample28	Sample4	Sample12	Sample20	Sample28
E	Sample5	Sample13	Sample21	Sample29	Sample5	Sample13	Sample21	Sample29	Sample5	Sample13	Sample21	Sample29
F	Sample6	Sample14	Sample22	Sample30	Sample6	Sample14	Sample22	Sample30	Sample6	Sample14	Sample22	Sample30
G	Sample7	Sample15	Sample23	PC	Sample7	Sample15	Sample23	PC	Sample7	Sample15	Sample23	PC
H	Sample8	Sample16	Sample24	NC	Sample8	Sample16	Sample24	NC	Sample8	Sample16	Sample24	NC

Note: It is very important that samples and controls are pipetted consecutively in the exact positions as shown in the above scheme. PC's should always be at positions G4, G8 and G12. NC's should always be at positions H4, H8 and H12. No duplicate sample names are allowed.

3. Vortex and spin down all DNA sample. Carefully open the tubes with the DNA solution one by one and avoid contamination of gloves and pipette. Using a new aerosol resistant pipette tip add 5 µl of each DNA sample (20 ng/µl) to the reaction tube/well containing the master mix.
   N.B. Replace gloves if suspected of contamination.

4. Using a new aerosol resistant pipette tip, add 5 µl of the positive and negative control (PC and NC respectively) to the designated reaction tubes/wells containing the master mix.

5. Seal the plate, spin down and take the plate to the Amplification Area.

6. Load the reaction plate into the real-time PCR instrument.

   Run protocols and templates for each machine and software version can be downloaded at https://microbenlab.shinyapps.io/CeliaScan/

7. Load the appropriate run protocol for your machine and software version, fill in the sample names and start your run.

A. On the **Roche Light Cycler 480 Instrument** use the following settings:

Pre-Incubation	
Polymerase activation	10 mins at 95ºC
Amplification	
Number of cycles	40 cycles
Denaturation	20 sec at 95ºC
Annealing	10 sec at 63ºC Second target at 60ºC with step size of 0.16ºC.
Extension	10 sec at 72ºC (acquisition mode single)
Melting Curve	
Denaturation	5 sec at 95ºC
Hold	1 min at 62ºC
Dissociation	97ºC Continuous at 25 acquisitions per ºC
Cooling	30 sec at 40ºC

Detection format must be set to SYBR Green I / HRM Dye

B. The following settings apply for:

Applied Biosystems® 7500 Real-Time PCR System
Applied Biosystems® StepOne and StepOnePlus Real-Time PCR Systems

For StepOne and StepOnePlus software use the "Advanced Setup" instead of the Setup Wizard.
If asked you can use "PC" as the reference sample and any of your SYBR targets as endogenous control.

Stage 1	
Polymerase activation	10 mins at 95ºC
Stage 2	
Number of cycles	15 cycles
Denaturation	20 sec at 95ºC
Annealing	10 sec at 63ºC Auto Increment at -0,2
Extension	10 sec at 72ºC
Stage 3	
Number of cycles	25 cycles
Denaturation	20 sec at 95ºC
Annealing	10 sec at 60ºC
Extension	30 sec at 72ºC (plate read on this step)
Stage 4 (Dissociation stage)	
Denaturation	15 sec at 95ºC
Hold	1 min at 62ºC
Dissociation	15 sec to 97ºC (plate read on this step)

Cooling	15 sec at 60ºC

SYBR detector must be used with Quencher and Passive Reference set to "none".
Note:
- In the software of your machine choose the appropriate instrument (e.g. 7500 Fast (96 Wells))
- select "Quantitation – Comparative Ct (ΔΔCt) as the experiment type
- select "SYBR® Green Reagents" and select "Include Melt Curve" checkbox
- select "Fast" as the ramping speed.

C. For the **Biorad CFX96™ and CFX96 Touch™ Real-Time PCR Detection Systems** use the following settings:

1. Polymerase activation	10 mins at 95ºC
2. Denaturation	20 sec at 95ºC
3. Annealing	10 sec at 63ºC Auto Increment at -0,2
4. Extension	10 sec at 72ºC
5. GOTO step2, 15 more times	
6. Denaturation	20 sec at 95ºC
7. Annealing	10 sec at 60ºC
8. Extension	30 sec at 72ºC (plate read on this step)
9. GOTO step 6, 25 more times	
10. Hold	1 sec at 62ºC
11. Dissociation	97ºC at 0.2ºC increment
12. Cooling	15 sec at 60ºC

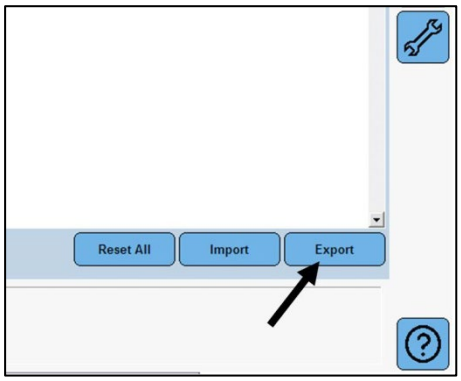
Note: Make sure the Scan Mode is set to "SYBR/FAM only"

5.3 Analysis of results

Analysis of the results is done by an online analysis tool. RAW data and plate setup files can be uploaded and are automatically and instantly translated into reports using a sophisticated algorithm. The generated reports can be downloaded from the server.
The next chapter will describe how to export the appropriate files on each platform.

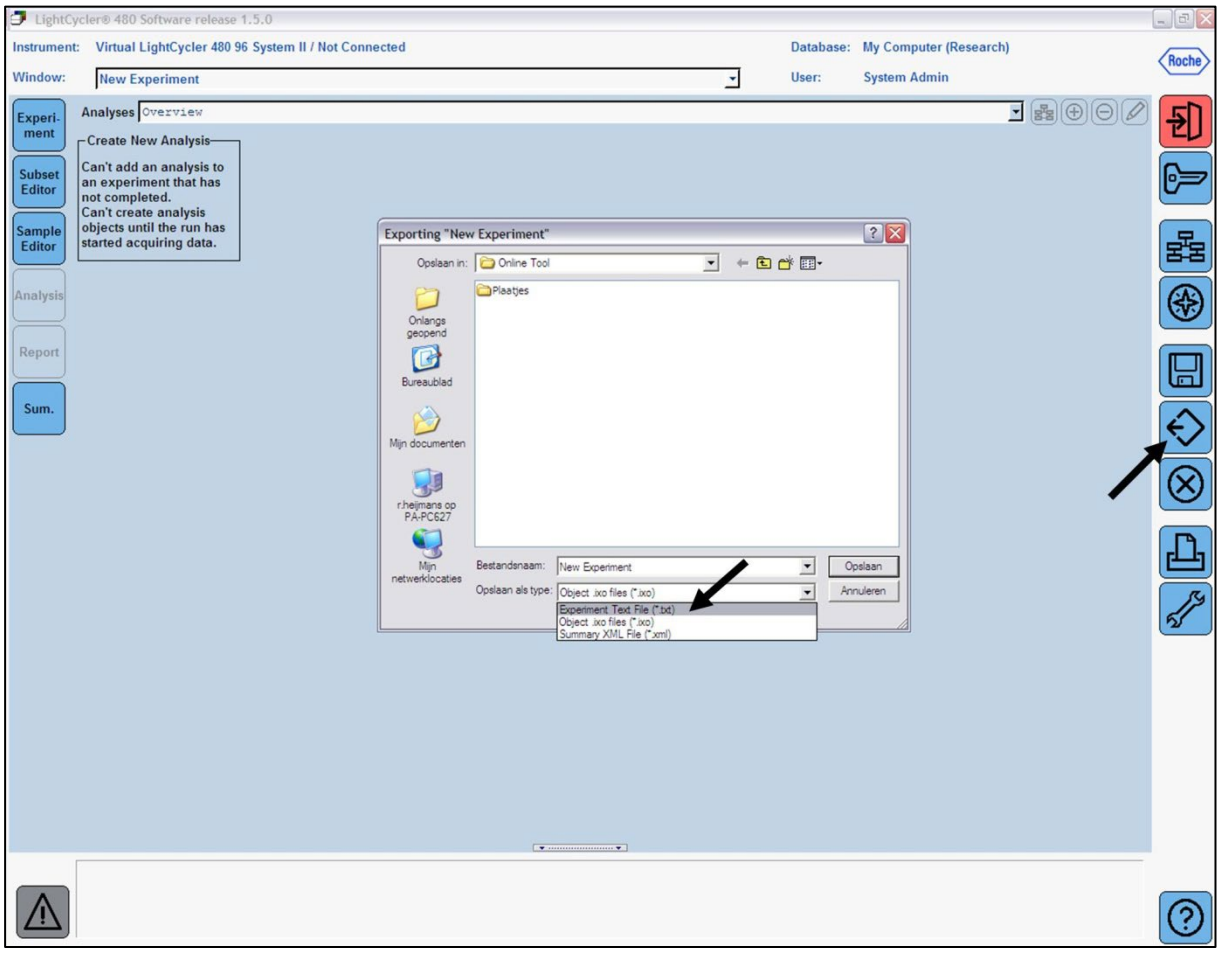
5.3.1 Exporting files on the Roche LightCycler® 480

To export the sample setup file on the Roche LightCycler480 do as follows:



On the “Sample Editor” tab click the “Export” button in the lower right corner to save the SETUP file.

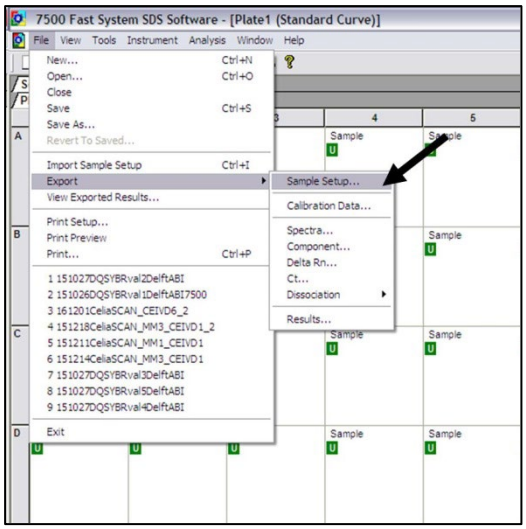
To export the raw data file on the Roche LightCycler480 do as follows:



On any tab click the “Export” button on the right, then select “Experiment text file” as output format and save the file.

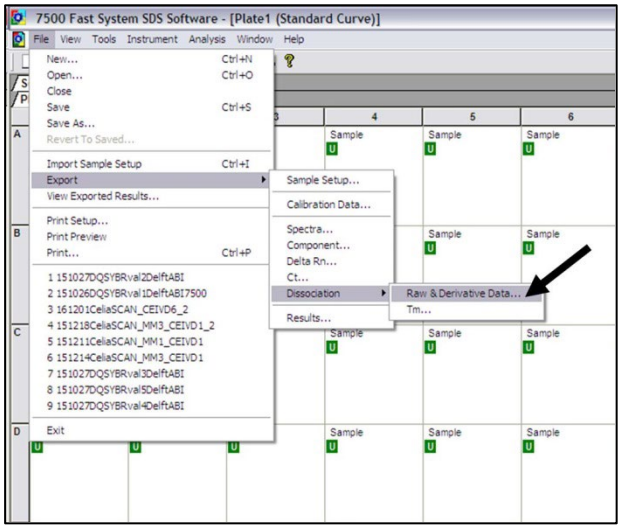
5.3.2 Exporting files on the Applied Biosystems® 7500, StepOne and StepOnePlus

To export the plate setup on the ABI7500 SDS software version 1.4 do as follows:



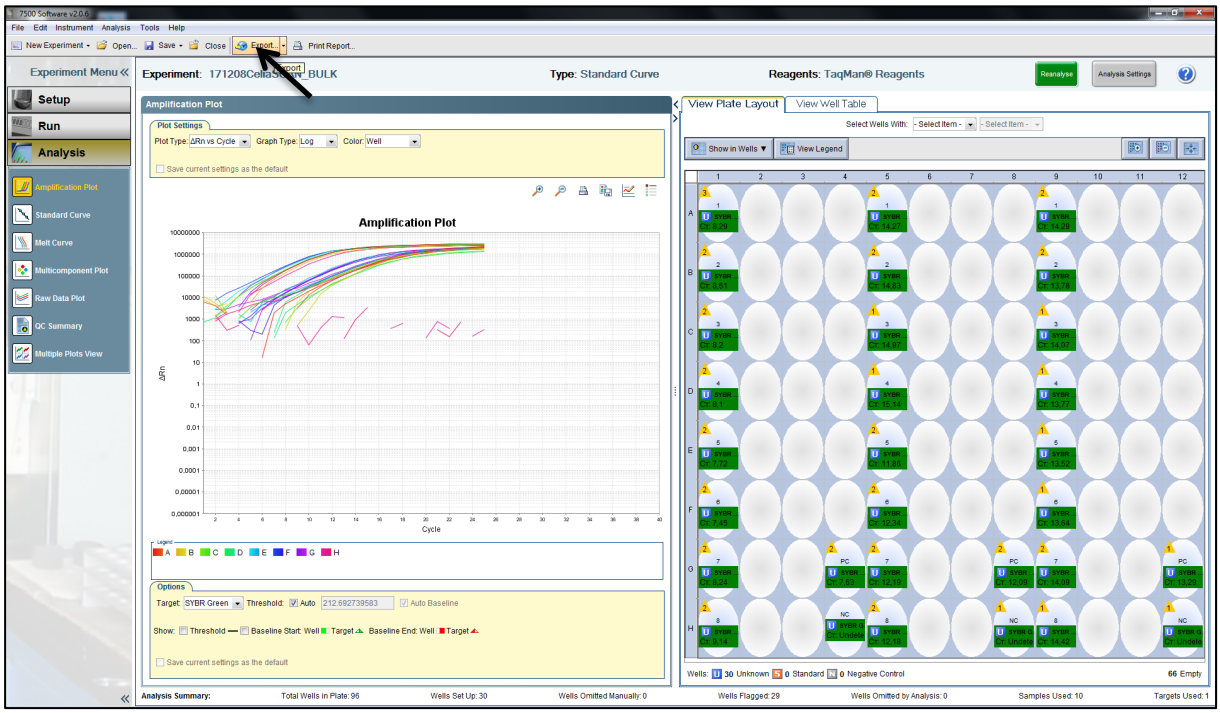
Select File → Export → Sample Setup and save the file.

To export the raw data file on the ABI7500 SDS software version 1.4 do as follows:

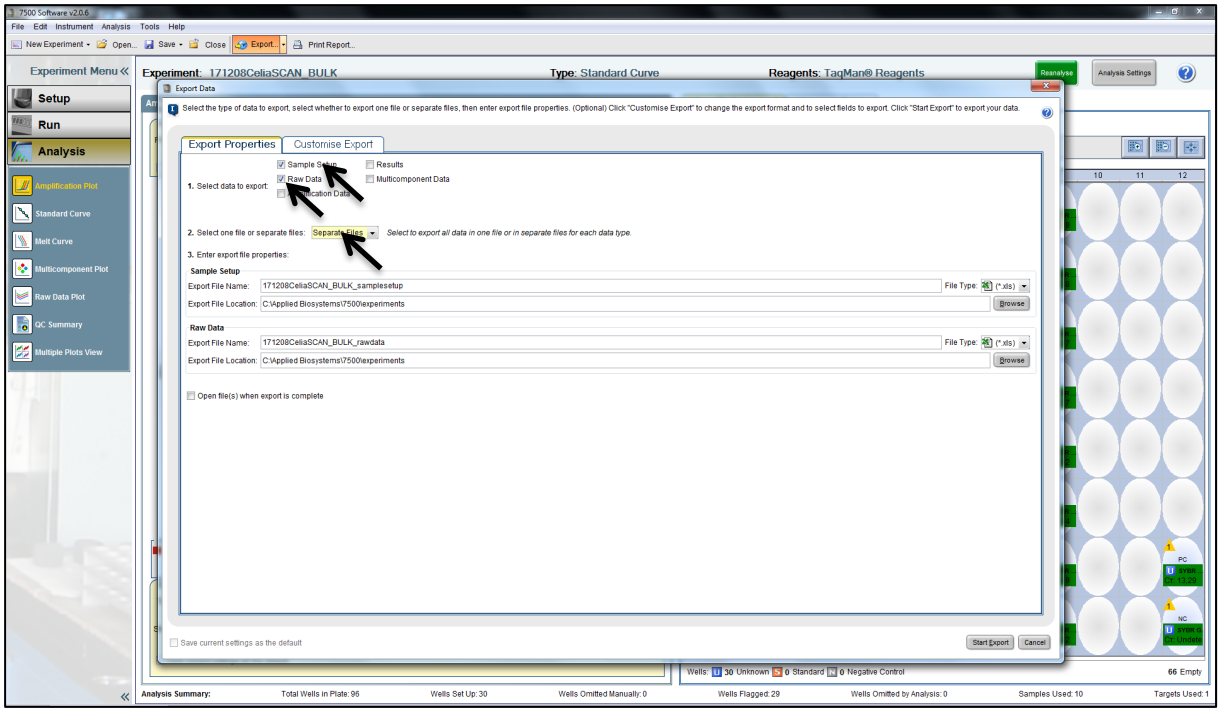


Select File → Export → Dissociation → Raw & Derivative Data and save the file.

To export the plate setup and RAW data on the ABI7500 SDS software version 2.0.6. and SDS software version 2.4 as well as the ABI StepOne and ABI StepOne Plus, do as follows:



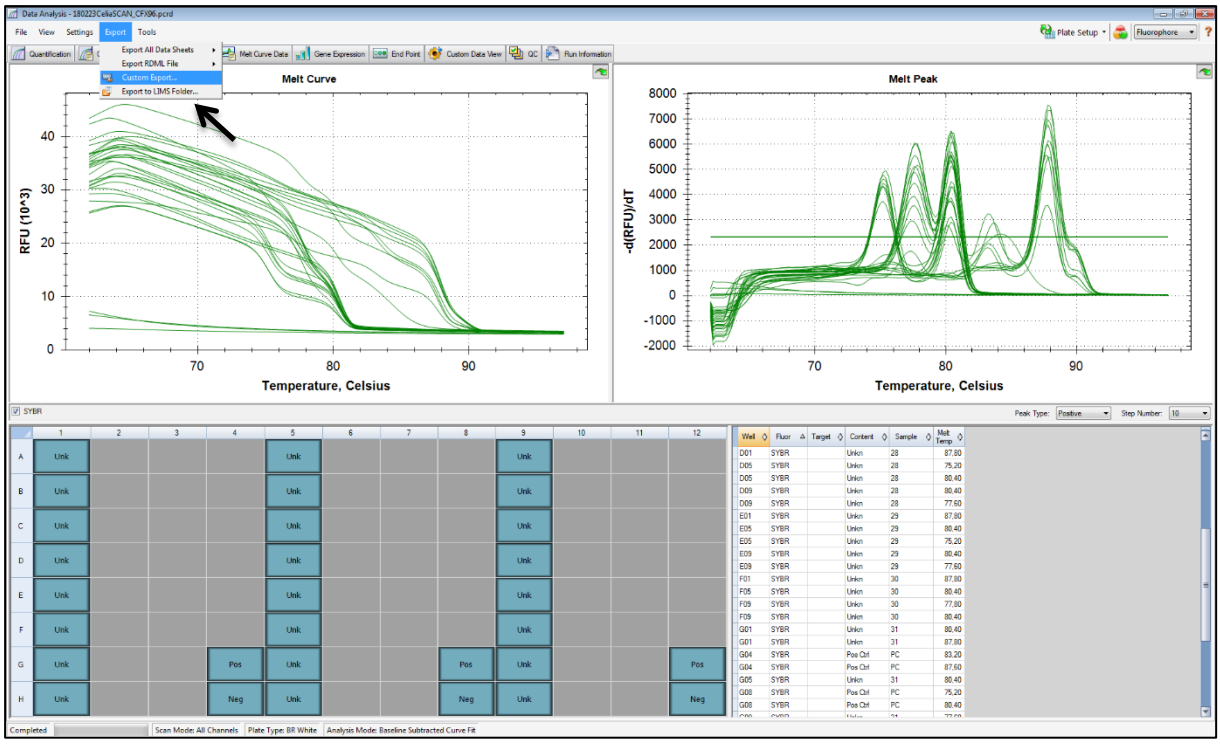
Click the “Export” button.



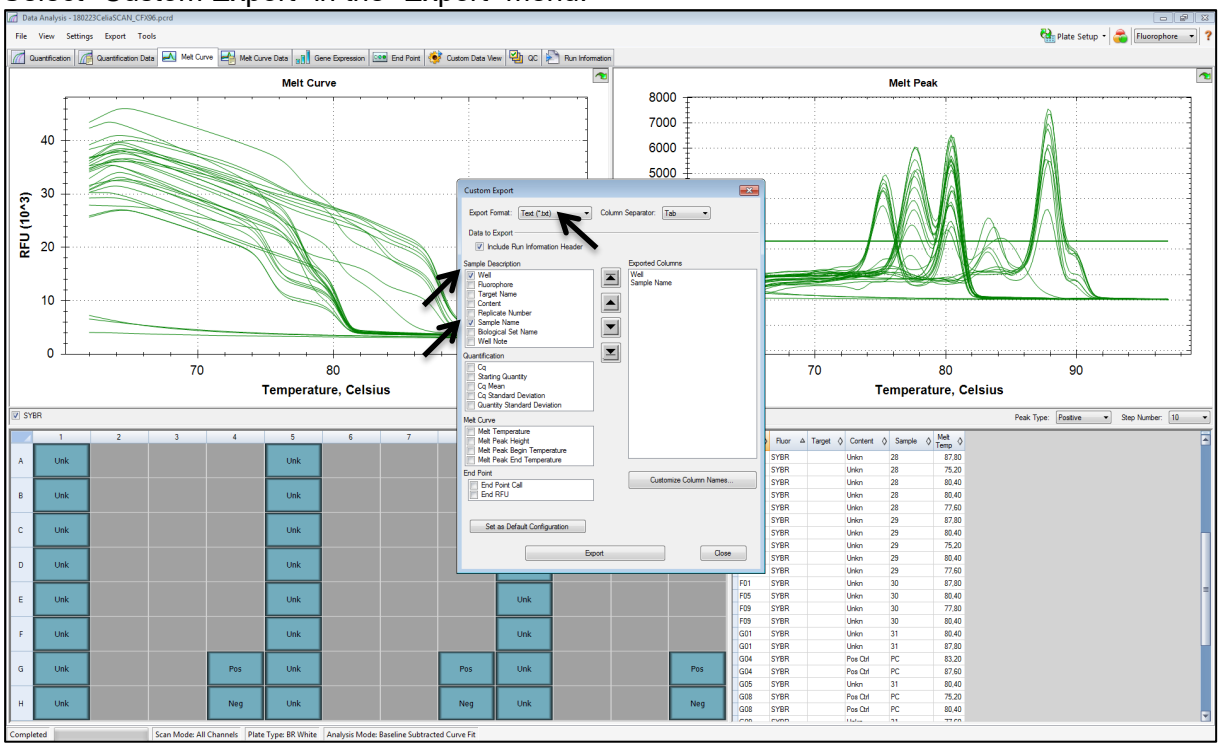
Select the “Sample Setup” and “Raw Data” checkboxes, make sure to select the export in separate files option and click “Start Export”.

5.3.3 Exporting files on the Bio-Rad CFX96

To export the plate setup on the BioRad CFX96 software version 3.1 do as follows:

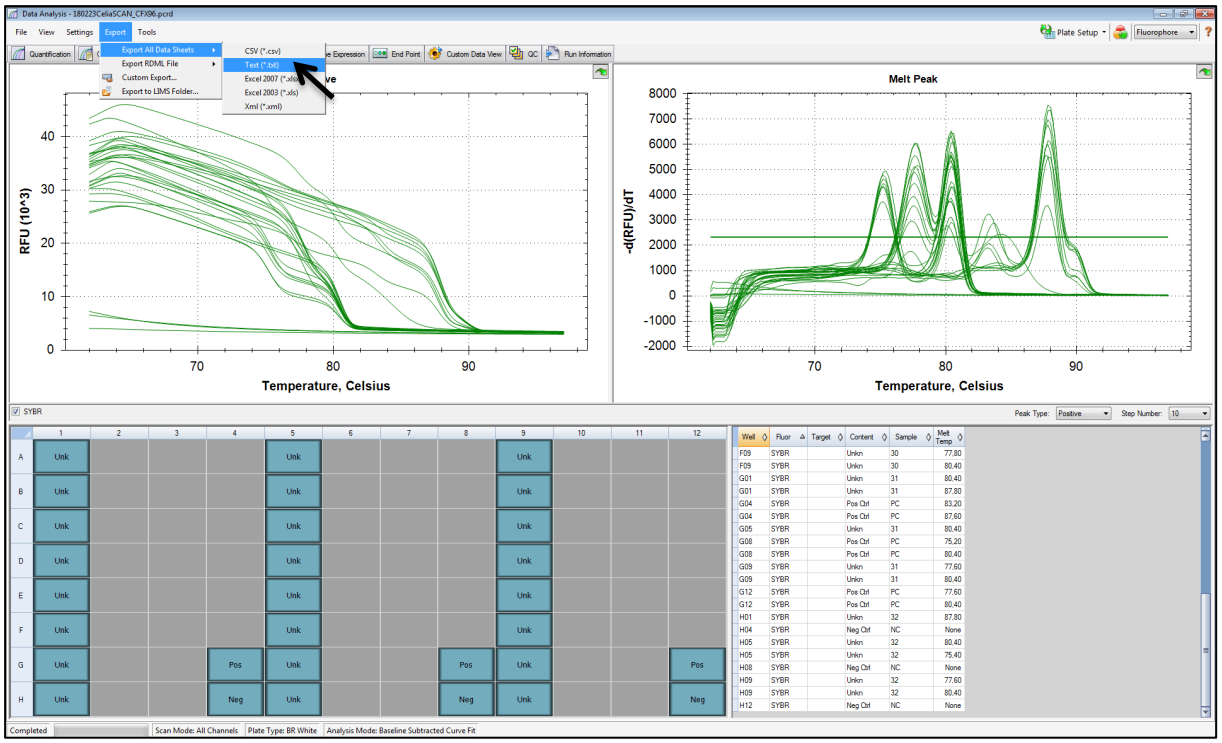


Select “Custom Export” in the “Export” menu.



Select the “Well” and “Sample Name” checkboxes, uncheck all other checkboxes and make sure the export format is set to Text (*.txt) and click the “Export” button.

To export the RAW data files on the BioRad CFX96 CFX Manager software version 3.1 do as follows:

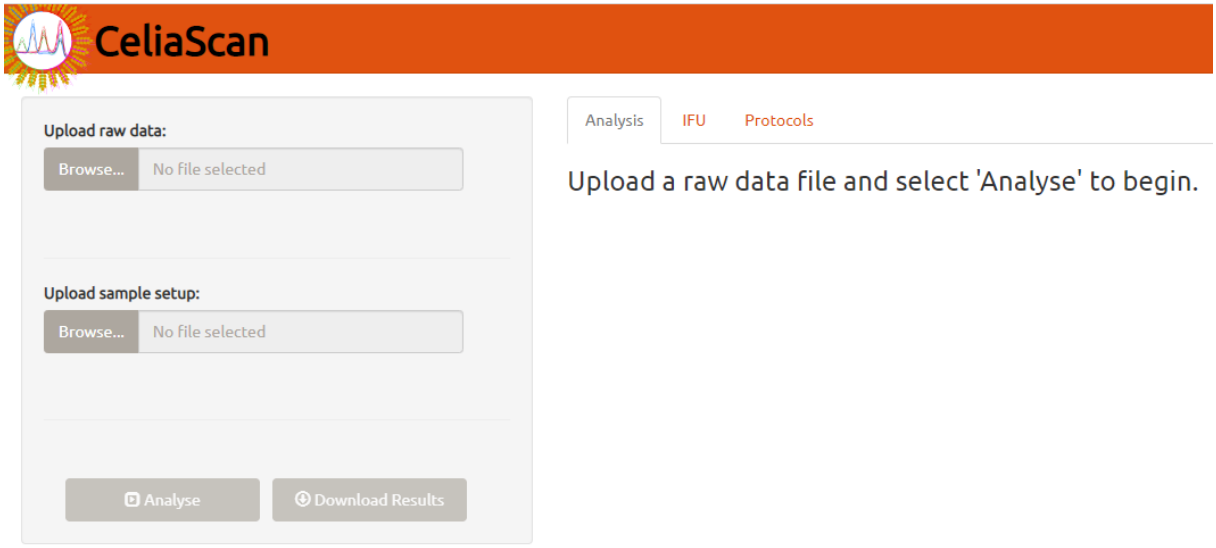


In the “Export” menu under “Select All Data Sheets” select the “Text (*.txt)” option. A list of output files is generated. The file named “experiment name - Melt Curve RFU Results_SYBR.txt” is the file containing the RAW data and should be used in the online analysis tool as the Data file.

5.3.4 Uploading and analysing the output files

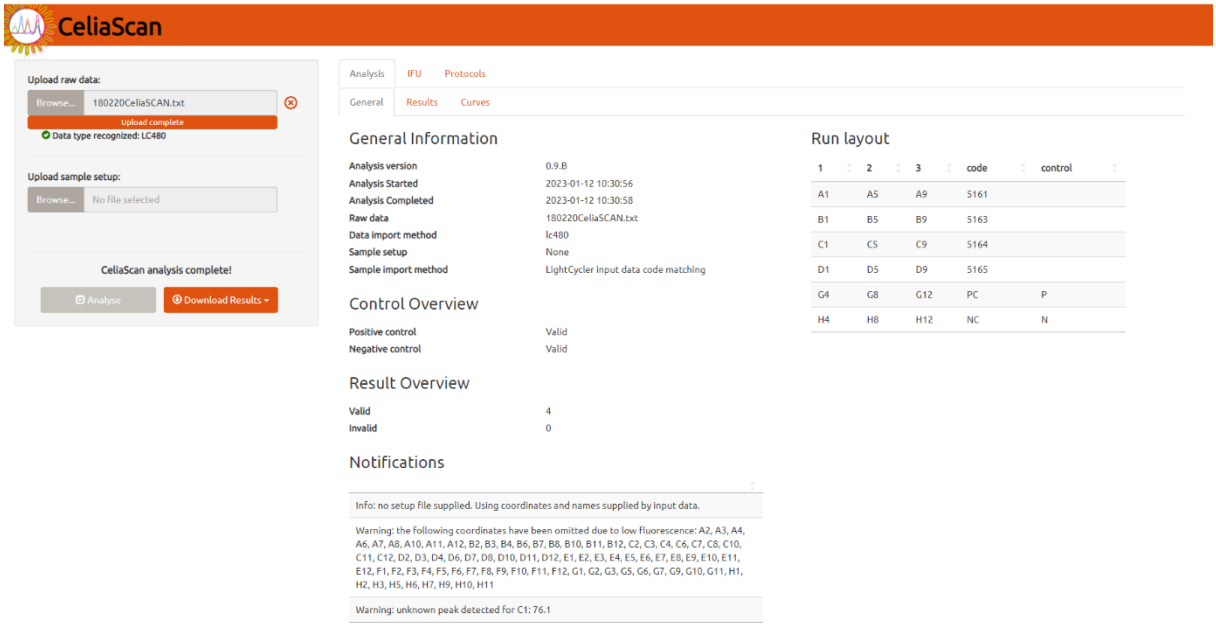
To analyse your results go to https://microbenlab.shinyapps.io/CeliaScan/

The procedure is as follows:
1.



Upload the raw data file. Next, upload the sample setup file. If no sample setup file is uploaded, the analysis will be based on plate coordinates. For the LightCycler 480, sample information is stored in the raw data file and therefore uploading a separate sample setup file is not necessary, however it may be done if desired. The software will automatically detect which Real-time PCR machine was used.

2.



After the analysis is done, general information about the run like run layout, validity of controls and notifications is shown on the General tab. On the Results tab, the haplotype and raw results are shown. On the Curves tab the meltcurve analyses are shown. To download your report click on Download Results.

5.4 Procedural notes

1. Use a unidirectional workflow in the laboratory (avoid returning to the pre-amplification area after working in the post-amplification area to minimize the risk of amplicon carry-over on clothing, hair and skin).

   Specimen Preparation area: Dedicated area to prepare the samples. All materials (equipment, supplies, protection, gloves, pipets, racks etc.) have to be dedicated to this area. Materials from this area may not be moved to the Pre-Amplification area.
   Pre-Amplification area: Dedicated area to prepare the reagents. All materials (equipment, supplies, protection, gloves, pipets, racks etc.) have to be dedicated to this area.
   Amplification area: Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, pipets, racks etc.) have to be dedicated to this area. Materials from this area, may not be moved to the Pre-Amplification Area, and may not be moved to the Specimen Preparation Area.

2. Always use aerosol resistant tips.
3. Be extremely careful when handling materials to prevent contamination. Always mix and spin down reagents and samples before opening. In case of any suspect of contamination, discard the materials.
4. Discard all consumed reagents upon completion of procedure in compliance with local bio hazardous waste regulations.
5. Careful analytical techniques and strict adherence to the directions in the assay procedure are essential to obtain reliable results.
6. Samples with equivocal results must be verified by repeat assays or isolation.
7. Do not pool reagents from different lots.
8. Avoid multiple freeze thaws, this will cause degradation of the CeliaSCAN assay kit components.
9. Spin down kit components in their vials before long-term storage.
10. All reagents can be used until the expiration date (printed on the labels).

6 Limitations of the procedure

- Optimal performance of the test requires good quality DNA and correct sample setup. (see 4.3. Specimen collection and preparation of DNA samples and 5.2. CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay kit test procedure).
- All instruments must be calibrated according to manufacturer's instructions.
- The CeliaSCAN assay has been validated for use with the Roche LightCycler ® 480 series and the Applied Biosystems® 7500 Real-Time PCR series. Do not use any other Real-Time PCR machines.
- Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination of reagents and/or DNA.
- The user should have a laboratory education in PCR techniques or have gained appropriate experience in the field of PCR techniques.
- CeliaSCAN is a low resolution HLA-DQ typing assay. Because of a large number of rare alleles, not all allele combinations can be resolved. Below is a list of all detectable alleles by CeliaSCAN.

HLA-DQA1	HLA-DQB1	HLA-F	HLA-DRA
DQA1*02:01:01:01 - DQA1*02:01:01:02	DQB1*02:01:01	F*01:01:01:01 - F*01:01:01:14	DRA*01:01:01:01 - DRA*01:01:01:03
DQA1*02:01:02	DQB1*02:01:02	F*01:01:02:01 - F*01:01:02:06	DRA*01:01:02
DQA1*03:01:01	DQB1*02:01:03 - DQB1*02:01:06	F*01:02	DRA*01:02:01 - DRA*01:02:03
DQA1*03:01:03	DQB1*02:01:07 - DQB1*02:01:08	F*01:03:01:01 - F*01:03:01:02	
DQA1*03:02:01:01 - DQA1*03:02:01:03	DQB1*02:01:10	F*01:04 - F*01:05	
DQA1*03:03:01:01 - DQA1*03:03:01:03	DQB1*02:01:11 - DQB1*02:01:12		
DQA1*03:03:02	DQB1*02:01:13 - DQB1*02:01:15		
DQA1*03:04	DQB1*02:01:16 - DQB1*02:01:17		
DQA1*05:01:01:01 - DQA1*05:01:01:03	DQB1*02:01:18 - DQB1*02:01:19		
DQA1*05:01:02	DQB1*02:01:20		
DQA1*05:03:01:01 - DQA1*05:03:01:02	DQB1*02:01:21 - DQB1*02:01:22		
DQA1*05:04	DQB1*02:01:23 - DQB1*02:01:24		
DQA1*05:05:01:01 - DQA1*05:05:01:10	DQB1*02:02:01:01 - DQB1*02:02:01:02		
DQA1*05:06:01:01 - DQA1*05:06:01:02	DQB1*02:02:02 - DQB1*02:02:04		
DQA1*05:07 - DQA1*05:09	DQB1*02:03 - DQB1*02:04		
DQA1*05:11	DQB1*02:05		
	DQB1*02:06		
	DQB1*02:07:01		
	DQB1*02:07:02		
	DQB1*02:08		
	DQB1*02:09		
	DQB1*02:10		
	DQB1*02:11 - DQB1*02:21		
	DQB1*02:23 - DQB1*02:25		
	DQB1*02:27 - DQB1*02:36		
	DQB1*02:38		
	DQB1*02:40 - DQB1*02:48		
	DQB1*02:50 - DQB1*02:55		
	DQB1*02:57 - DQB1*02:61		
	DQB1*02:63 - DQB1*02:69		
	DQB1*02:71 - DQB1*02:84		
	DQB1*02:86 - DQB1*02:90		
	DQB1*02:92 - DQB1*02:94		
	DQB1*02:96N - DQB1*02:101		
	DQB1*03:02:01:01 - DQB1*03:02:01:04		
	DQB1*03:02:02 - DQB1*03:02:09		
	DQB1*03:02:11 - DQB1*03:02:13		
	DQB1*03:02:15 - DQB1*03:02:21		
	DQB1*03:02:23 - DQB1*03:02:25		
	DQB1*03:05:01 - DQB1*03:05:04		
	DQB1*03:08		
	DQB1*03:11		
	DQB1*03:18		
	DQB1*03:32		
	DQB1*03:37		
	DQB1*03:45		
	DQB1*03:61 - DQB1*03:64		
	DQB1*03:67 - DQB1*03:68		
	DQB1*03:70		
	DQB1*03:80 - DQB1*03:81		
	DQB1*03:85		
	DQB1*03:106 - DQB1*03:107		
	DQB1*03:125		
	DQB1*03:132		
	DQB1*03:138		
	DQB1*03:14:01 - DQB1*03:14:02		
	DQB1*03:146		
	DQB1*03:153		
	DQB1*03:174 - DQB1*03:175		
	DQB1*03:178 - DQB1*03:179		
	DQB1*03:181		
	DQB1*03:184 - DQB1*03:185		
	DQB1*03:189 - DQB1*03:190		
	DQB1*03:199		
	DQB1*03:203 - DQB1*03:204		
	DQB1*03:210 - DQB1*03:211		
	DQB1*03:213N		
	DQB1*03:215		
	DQB1*03:220 - DQB1*03:226		
	DQB1*03:228 - DQB1*03:229		
	DQB1*03:233		
	DQB1*03:237N		
	DQB1*03:240		
	DQB1*03:245		
	DQB1*03:247		
	DQB1*03:250 - DQB1*03:251		
	DQB1*03:261 - DQB1*03:263		
	DQB1*03:265		
	DQB1*03:269N		
	DQB1*06:63		
	DQB1*06:139		

Table 1: List of all alleles detectable by CeliaSCAN. IPD-IMGT/HLA Release 3.30.0, 2017-10-01.

7 Performance characteristics

Analytical specificity

Cross-reactivity and interference studies were lead to determine whether CeliaSCAN is able to detect and measure specifically the intended targets in the presence of all known DQ haplotypes other than HLA-DQ2.5, HLA-DQ2.2 or HLA-DQ8 individually. These haplotypes are HLA-DQ2.3, HLA-DQ4.2, HLA-DQ4.3, HLA-DQ5.1, HLA-DQ5.2, HLA-DQ5.3, HLA-DQ6.1, HLA-DQ6.2, HLA-DQ6.3, HLA-DQ6.4, HLA-DQ7.3, HLA-DQ7.4/HLA-DQ7.6, HLA-DQ7.5, HLA-DQ9.2 and HLA-DQ9.3. Each sample is confirmed by SSCP.

Sample	HLA-DQ2.5	HLA-DQ2.5 Homozygous	HLA-DQ2.2	HLA-DQ8	Additional Information	Conlusion
DQ2.3	NO	NO	NO	NO	CARRIER OF THE HLA-DQB1*02 ALLELE (NON DQ2.5)	**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ4.2	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ4.3	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ5.1	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ5.2	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ5.3	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ6.1	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ6.2	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ6.3	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ6.4	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ7.3	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ7.4 DQ7.6	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ7.5	NO	NO	NO	NO	CARRIER OF THE HLA-DQA1*05 ALLELE (NON DQ2.5)	**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ9.2	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
DQ9.3	NO	NO	NO	NO		**NON HLA-DQ2.5 / NON HLA-DQ8**
PC	NO	NO	NO	NO		
NC	NO	NO	NO	NO		

Table 2: Cross-Reactivity results

Table 1 shows that none of the haplotypes result in HLA-DQ2.5, HLA-DQ2.2 or HLA-DQ8 positivity.

Precision

The table below shows the precision as well as the ANOVA Gage R&R analysis including SD and CV(%)

MasterMix1

Target Allele	Repeatability	Reproducibility	Part-to-Part	Total Gage R&R	Total Gage R&R (%)	Total Variation	SD	CV (%)
1	0,0704	0,0718	2,7621	0,1422	4,8961	2,9043	0,0356	0,0454
2	0,0606	0,0730	2,7669	0,1336	4,6071	2,9005	0,0312	0,0383
3	0,0391	0,0760	2,7790	0,1151	3,9769	2,8941	0,0301	0,0356
4	0,0761	0,0711	2,7595	0,1472	5,0651	2,9067	0,0257	0,0289

MasterMix2

Target Allele	Repeatability	Reproducibility	Part-to-Part	Total Gage R&R	Total Gage R&R (%)	Total Variation	SD	CV (%)
1	0,1024	0,0684	2,7484	0,1707	5,8491	2,9192	0,0311	0,0408
2	0,0625	0,0728	2,7659	0,1353	4,6629	2,9012	0,0365	0,0448
3	0,0581	0,0733	2,7682	0,1314	4,5320	2,8996	0,0308	0,0362

MasterMix3

Target Allele	Repeatability	Reproducibility	Part-to-Part	Total Gage R&R	Total Gage R&R (%)	Total Variation	SD	CV (%)
1	0,0714	0,0717	2,7616	0,1430	4,9246	2,9047	0,0231	0,0294
2	0,0478	0,0747	2,7738	0,1225	4,2301	2,8963	0,0310	0,0380

Table 3: Precision

Accuracy

The CeliaSCAN assay was evaluated in a clinical validation study at two different sites. Positive and negative EDTA-blood samples from VUMC (n=85 MMI +20 PA) and Reinier Haga MDC (n=61) were genotyped for HLA-DQA1 and HLA-DQB1 by SSCP/HD method at VUMC. These samples were then genotyped with CeliaSCAN (VUmc). The results were fully concordant (n=166).

List of symbols as used in labeling

CE	Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices
IVD	*In vitro* diagnostic medical device
	Manufacturer
LOT	Batch code (Lot)
	Use by date
	Temperature Limitation
Σ	Contains sufficient for <n> tests

List of Abbreviations

CD	Celiac Disease
CE	Conformité Européenne
DNA	Deoxyribonucleic acid
HLA	Human Leucocyte Antigen
IVD	In Vitro Diagnostics
MHC	Major Histocompatibility Complex
ng/µl	Nanograms per microliter
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism
Tm	Melting Temperature

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