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

Index

1. Intended use

The CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay is intended to be used for in vitro detection of the MHC class II HLA-DQ2.5 (hetero- and homozygous), HLA-DQ8, HLADQ2.2 and HLA-DQA1*05 markers in human DNA, with the aim to exclude Celiac disease as a diagnosis. There is no need of any specific information to be provided. The CeliaSCAN Assay is a non-automated qualitative assay, meaning it detects whether the above mentioned alleles are present or not. CeliaSCAN uses genomic DNA derived from any human sample using any DNA isolation method as input material and is suitable for all populations and not intended for companion diagnostics.

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.

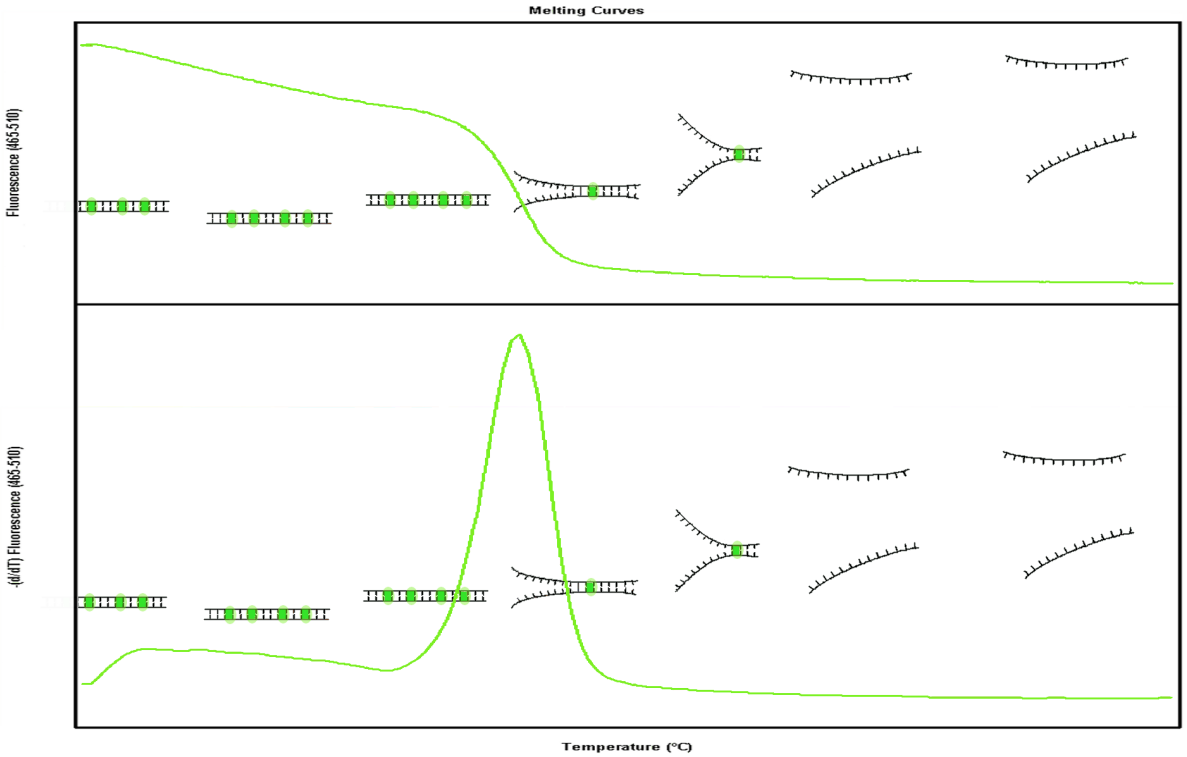


Figure 1: Melting curve analysis principle

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 Materials provided with each CeliaSCAN 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 HLA-DQA1*02, HLA-DQA1*03, HLA-DQB1*02 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 HLA-DQA1*05, HLA-DQB1*0302 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 HLA-DQ2.5 homozygous SNP 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, a synthetic double stranded oligonucleotide containing the DNA sequences for HLA-DQA1*02, HLA-DQA1*03, HLA-DQB1*02 and the HLA-F control.

   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, a synthetic double stranded oligonucleotide containing the DNA sequences for HLA-DQA1*05, HLA-DQB1*0302 and the HLA-DRA control.

   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, a double stranded oligonucleotide containing the DNA sequences for the HLA-DQ2.5 homozygous SNP and the HLA-DRA control.

   Positive Control MM3

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

Further details on material safety and hazard indications are described in Annex I: Material Safety Data Sheet, of this document.

4.2 Additional required materials and equipment

PCR amplification can be carried out on the Roche LightCycler® 480, the Applied Biosystems® 7500 Real-Time PCR Systems, the Applied Biosystems® QuantStudio™ 5 Real-Time PCR system and the BioRad CFX96 Real-Time PCR detection system.

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

- Filter Tips
- 96-well PCR plate
- Optical Seal

4.3 Additional user training

When desired an additional CeliaSCAN user training can be requested at Microbe&Lab. A dedicated technician will train personal on site or via a teams call. To apply for a user training, please send an email to laboratorium@microbenlab.com

4.4 Specimen collection and preparation of DNA samples

The CeliaSCAN HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 Assay is intended to be used on genomic DNA samples. Commercial DNA extraction kits, such as the Roche High Pure PCR Template Preparation Kit, can be used to obtain genomic DNA from any human cell specimen. No commercial DNA extraction kit is provided with CeliaSCAN.

For optimal performance it is recommended that DNA samples are diluted to a concentration of 20 ng/µl.

4.5 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. SYBR green is light sensitive, so store all kits and mastermixes in a dark area when not in use.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. Use of reagents past the expiry date is not recommended since correct results can not be guaranteed.

4.6 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. The procedure may only be carried out by trained laboratory personnel. Any deviations from this procedure may result in failure of the assay or incorrect results.

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 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.

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 www.microbe-lab.com/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 and QuantStudio™ 5 Real-Time PCR Systems

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™ 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

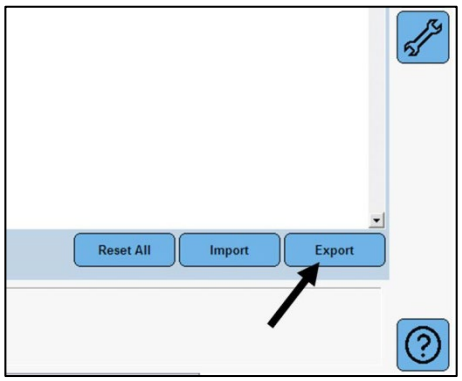
Note: Make sure the Scan Mode is set to "SYBR/FAM only"

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.

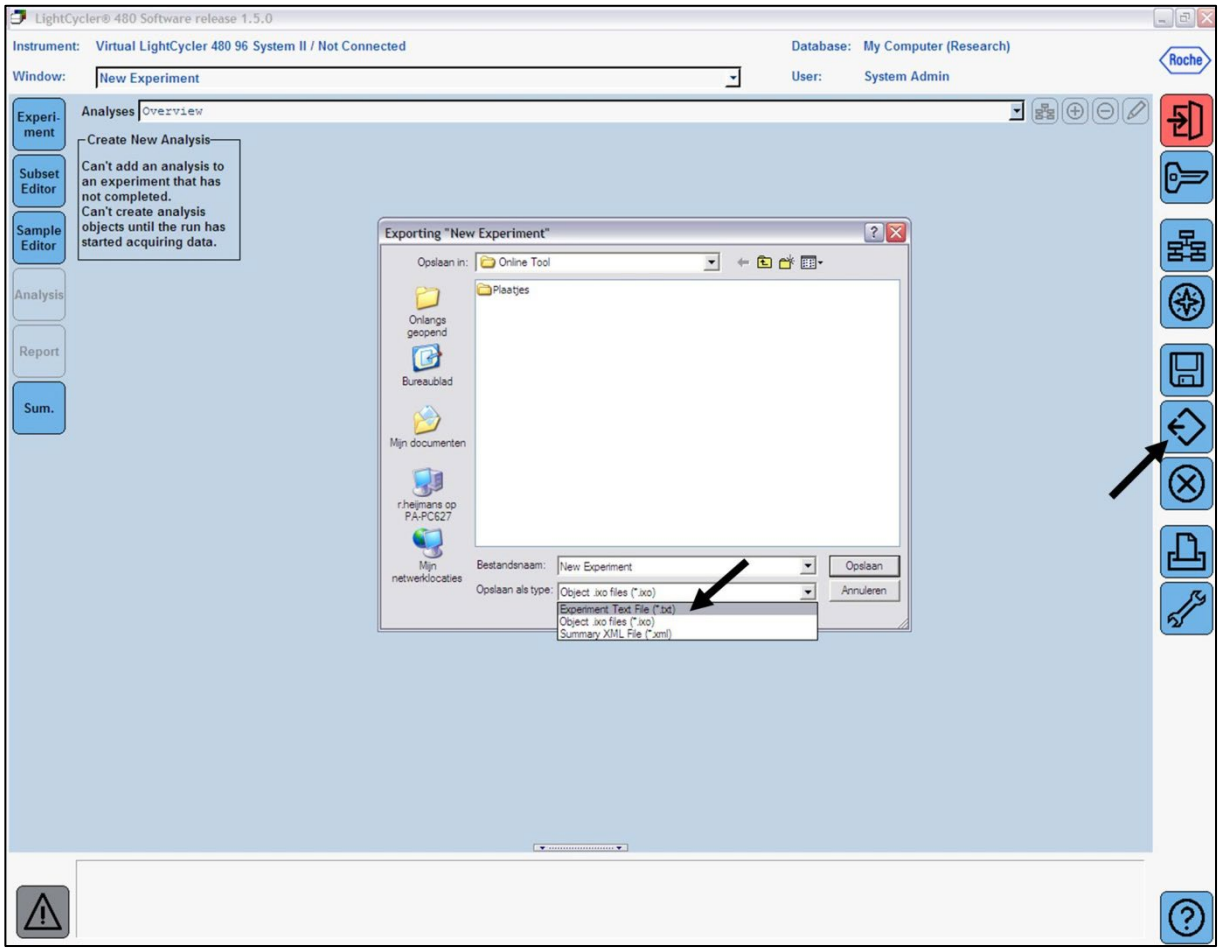
5.3.1 Exporting files on the Roche LightCycler® 480

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.

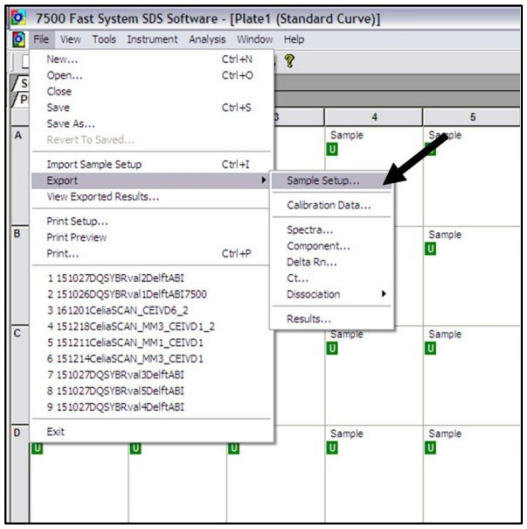
To export the raw data file on the Roche LightCycler480 do as follows:



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

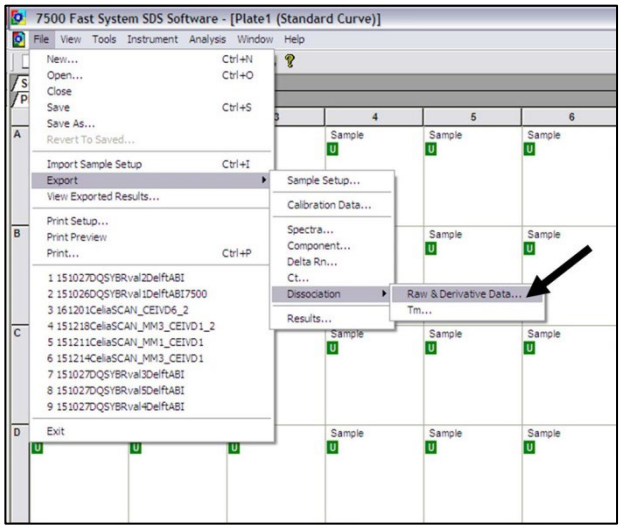
5.3.2 Exporting files on the Applied Biosystems® 7500

To export the plate setup on the ABI7500 SDS software versions 1.4 and 1.5 do as follows:



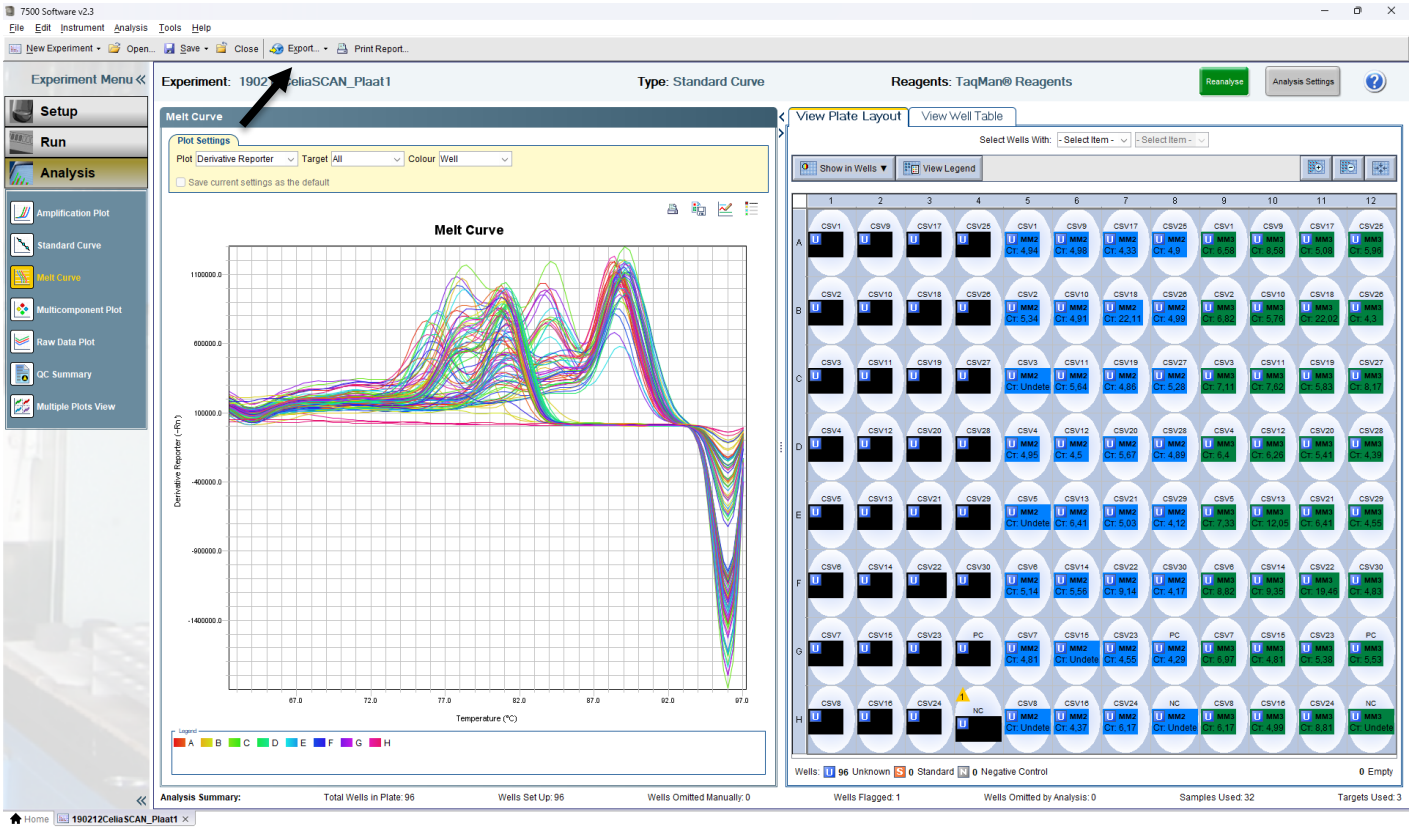
Select File → Export → Sample Setup and save the file.

To export the raw data file on the ABI7500 SDS software version 1.4 and 1.5 do as follows:

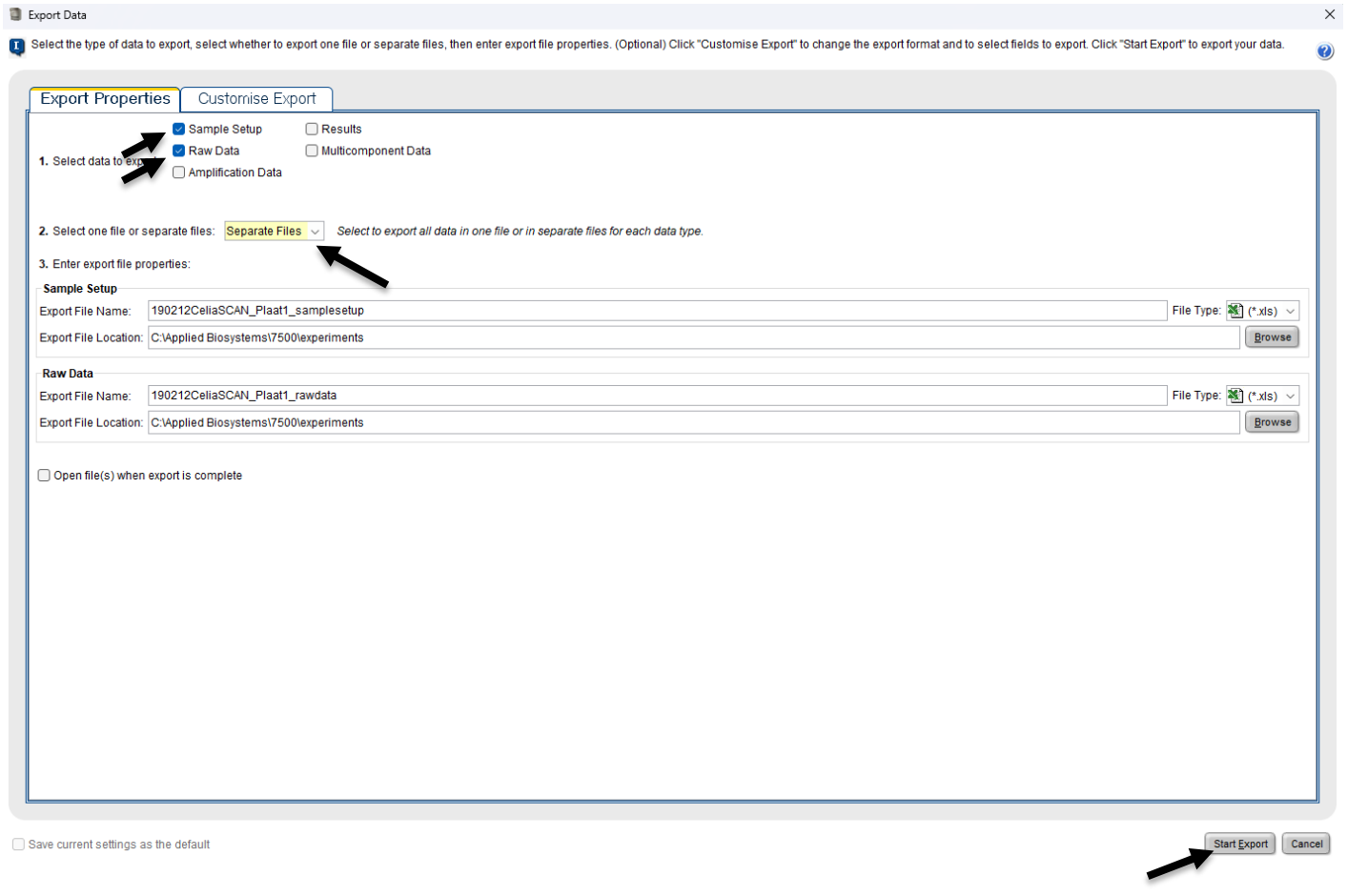


Select File → Export → Dissociation → Raw & Derivative Data and save the file.

To export the plate setup on the ABI7500 SDS software version 2.3 do as follows:



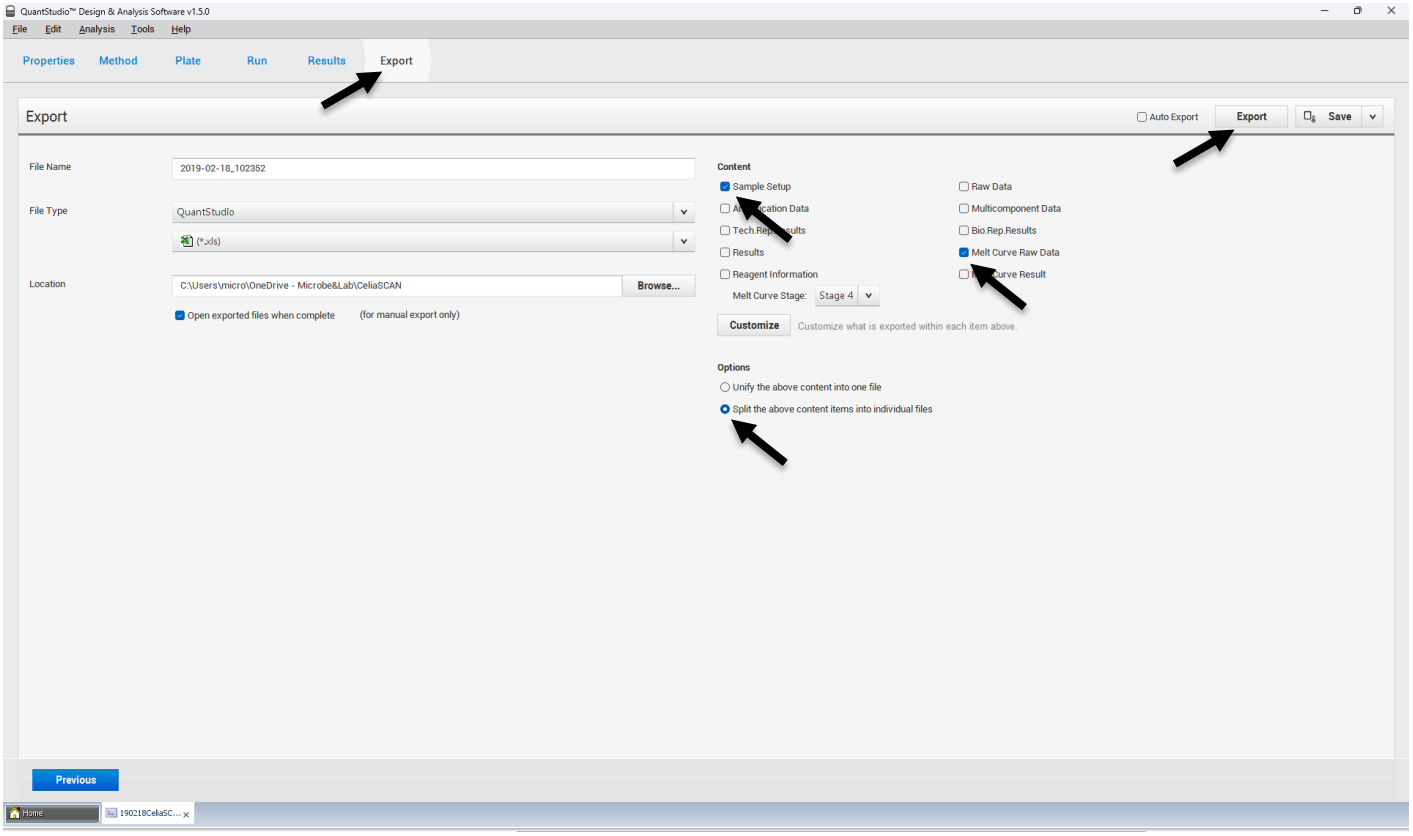
Click the “Export” button.



Under section 1, select “Sample Setup” and “Raw Data”, under section 2 select “Separate Files” and under section 3 select the export file names and locations. Click “Start Export”.

5.3.3 Exporting files on the Applied Biosystems® QuantStudio™ 5

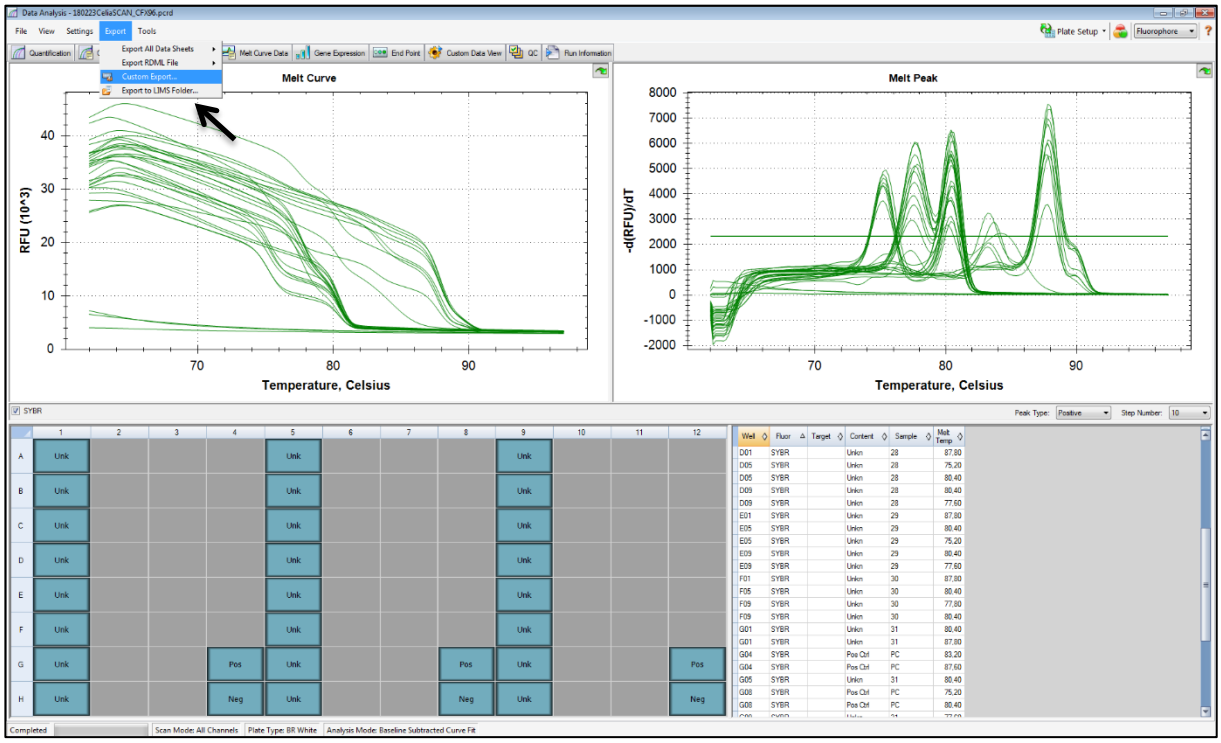
To export the plate setup as well as the raw data file on the Applied Biosystems® QuantStudio™ 5 Design & Analysis Software version 1.5.0 do as follows:



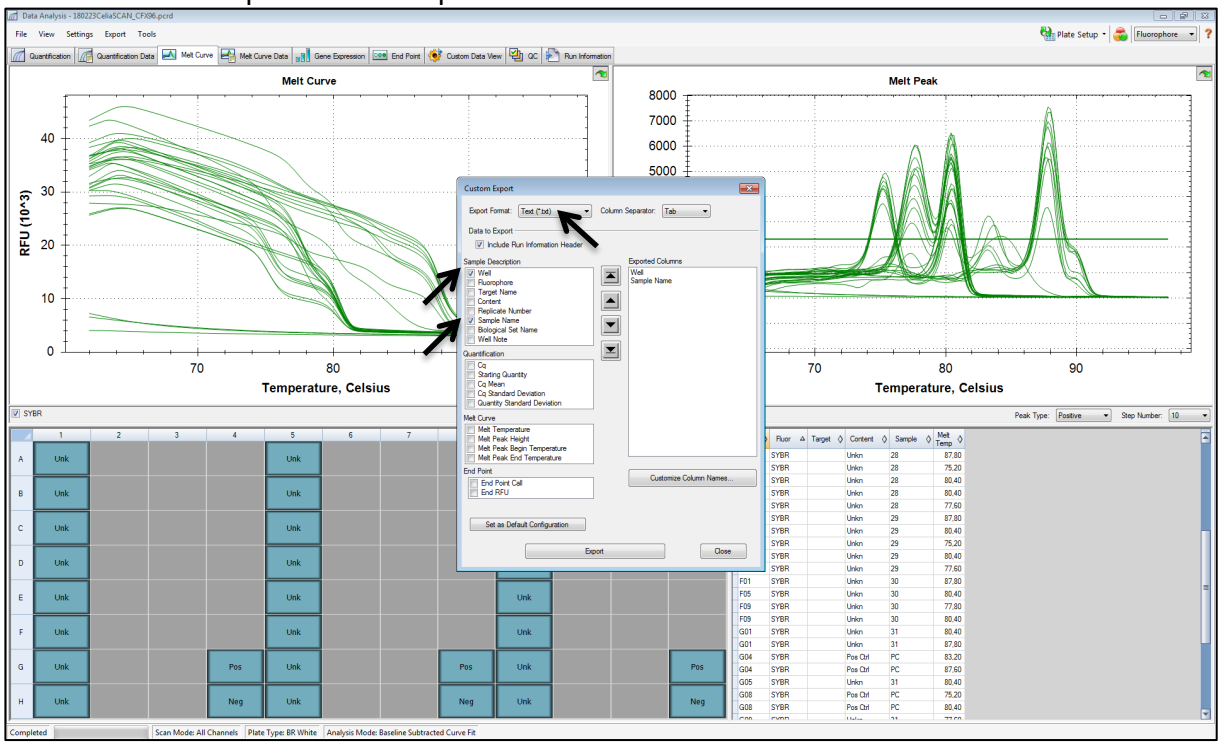
On the export tab under “Content” select “Sample Setup” and “Melt Curve Raw Data”. Under “Options” select "Split the above content items into individual files”. On the left select the export file name and location and click on “Export” in the upper right corner.

5.3.4 Exporting files on the Bio-Rad CFX96

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

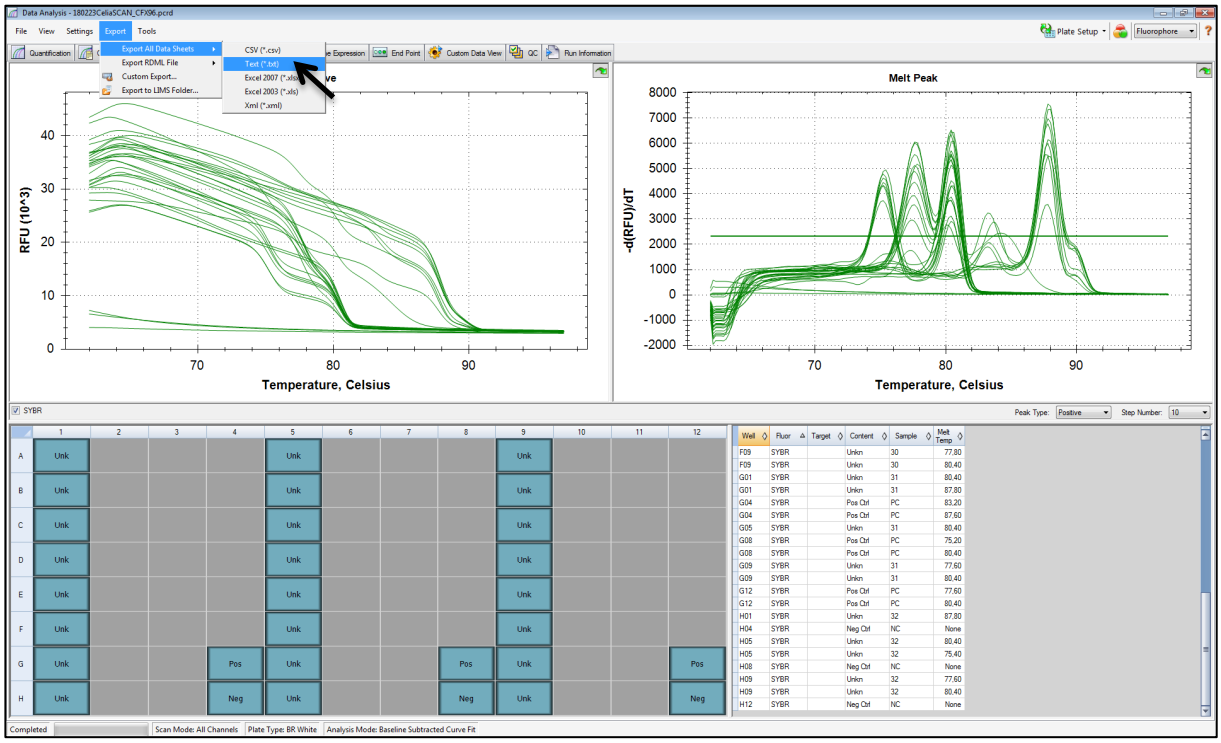


Select “Custom Export” in the “Export” menu.



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:

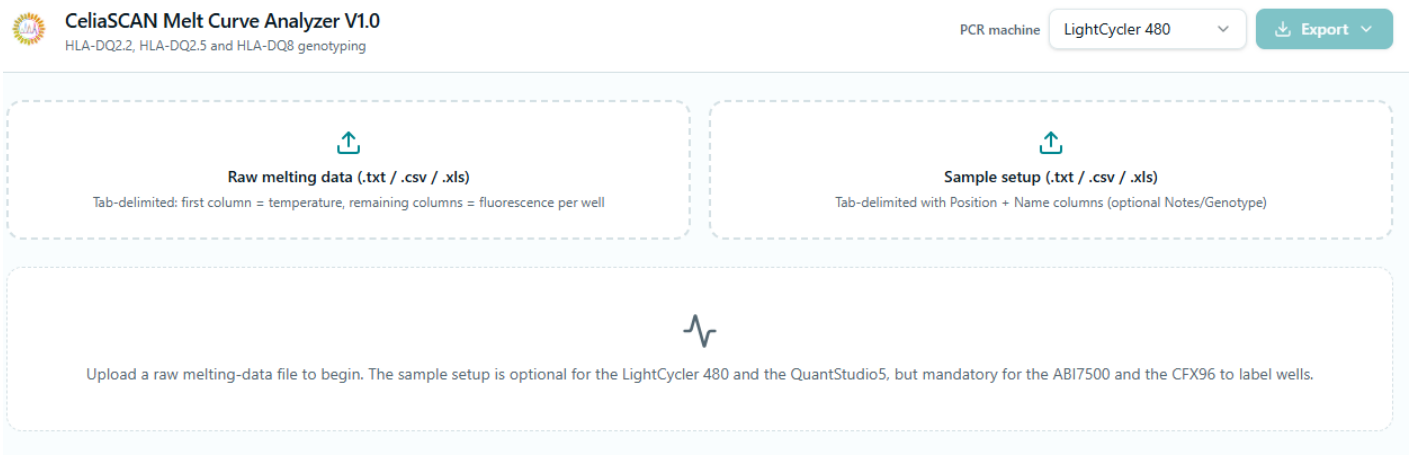


In the “Export” menu under “Select All Data Sheets” select the “Text (*.txt)” option. A list of output files is generated. The file named “experiment name - Melt Curve RFU Results_SYBR.txt” is the file containing the RAW data and should be used in the online analysis tool as the Data file.

5.3.5 Uploading and analysing the output files

To analyse your results, go to https://celiascan.lovable.app/
The procedure is as follows:

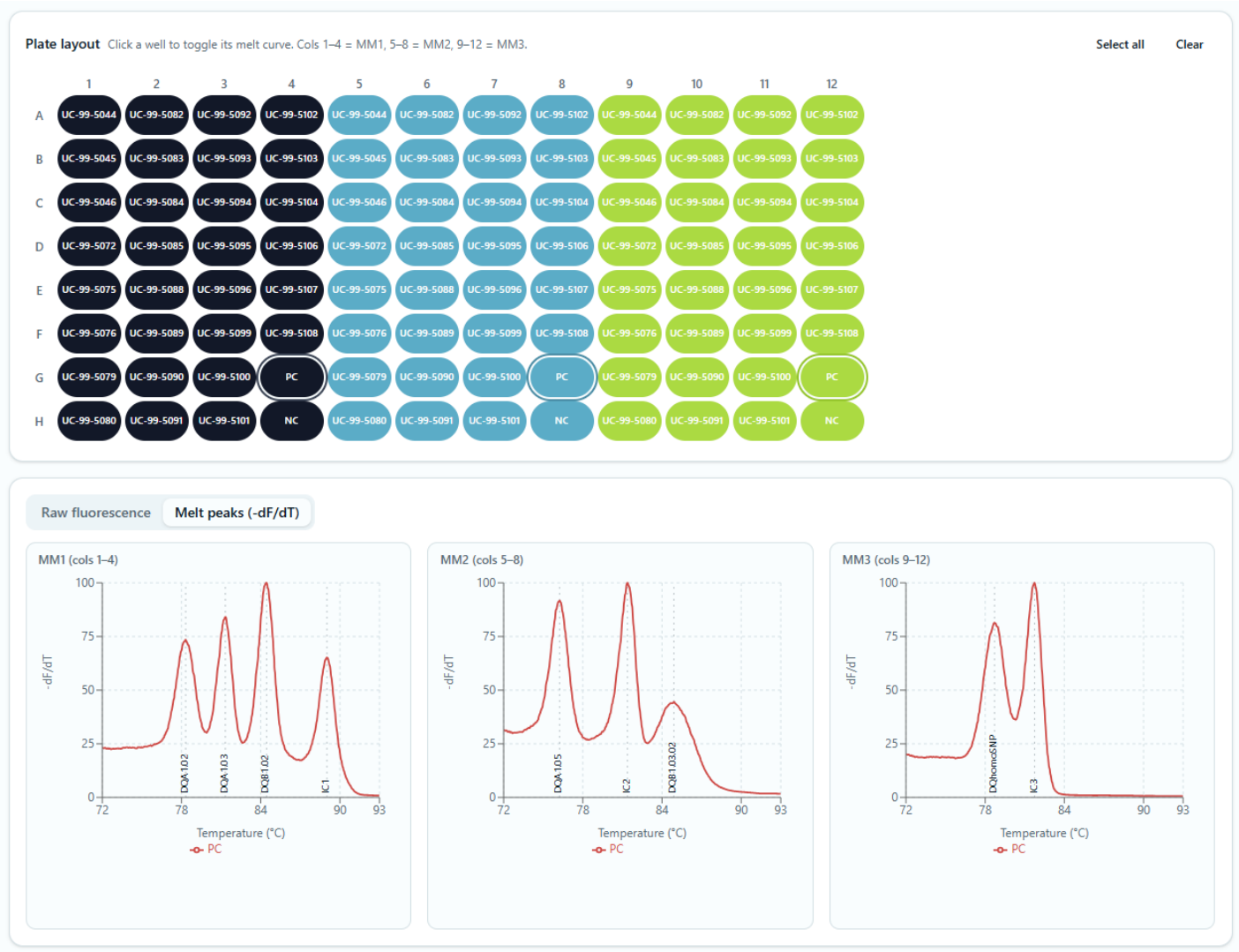
1.



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, the Run quality control section shows whether the positive and negative controls are valid.

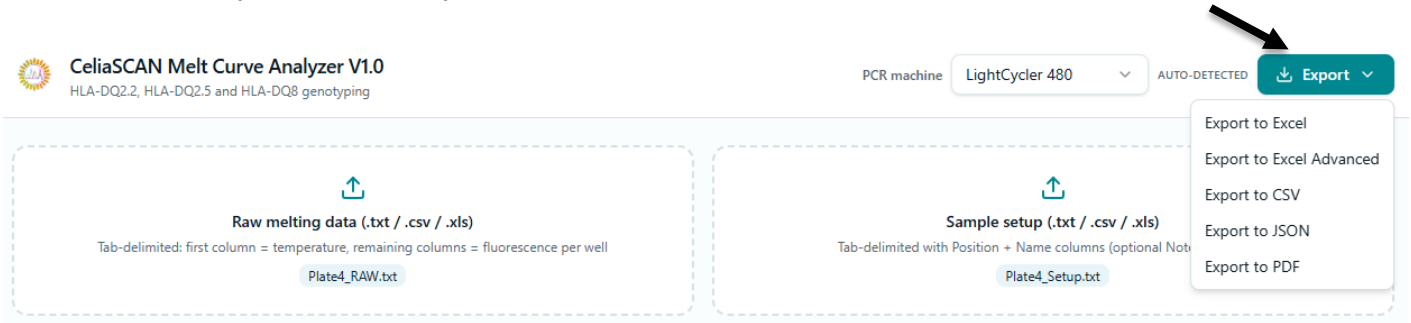


Below the Run quality control section is the interactive Plate layout section. To view the melting plots for a specific sample or control, simply click on the location on the plate layout and all three melting plots belonging to that sample or control will be shown below, multiple samples can be selected at once.

Main results MM1 = cols 1–4, MM2 = cols 5–8, MM3 = cols 9–12. Samples are grouped across the three MMs.

Sample	Name	Wells	DQA1.02	DQA1.03	DQB1.02	IC1	DQA1.05	IC2	DQB1.03.02	DQhomoSNP	IC3	Primary	Secondary
A1	UC-99-5044	A1 / A5 / A9	—	—	—	✓	—	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	
B1	UC-99-5045	B1 / B5 / B9	—	✓	—	✓	—	✓	✓	✓	✓	HLA-DQ8	
C1	UC-99-5046	C1 / C5 / C9	—	—	✓	✓	✓	✓	—	✓	✓	HLA-DQ2.5	
D1	UC-99-5072	D1 / D5 / D9	—	✓	—	✓	✓	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
E1	UC-99-5075	E1 / E5 / E9	—	✓	—	✓	✓	✓	✓	✓	✓	HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
F1	UC-99-5076	F1 / F5 / F9	—	—	✓	✓	✓	✓	—	✓	✓	HLA-DQ2.5	
G1	UC-99-5079	G1 / G5 / G9	—	—	—	✓	✓	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
H1	UC-99-5080	H1 / H5 / H9	—	—	—	✓	✓	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
A2	UC-99-5082	A2 / A6 / A10	—	—	—	✓	✓	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
B2	UC-99-5083	B2 / B6 / B10	—	—	—	✓	✓	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	Carrier of the HLA-DQA1*05 allele (Non DQ2.5)
C2	UC-99-5084	C2 / C6 / C10	✓	—	✓	✓	—	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	HLA-DQ2.2
D2	UC-99-5085	D2 / D6 / D10	—	✓	—	✓	—	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	
E2	UC-99-5088	E2 / E6 / E10	—	—	✓	✓	✓	✓	—	—	✓	HLA-DQ2.5 Homozygous	
F2	UC-99-5089	F2 / F6 / F10	—	—	✓	✓	✓	✓	—	✓	✓	HLA-DQ2.5	
G2	UC-99-5090	G2 / G6 / G10	✓	—	—	✓	—	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	
H2	UC-99-5091	H2 / H6 / H10	✓	✓	—	✓	—	✓	—	✓	✓	Non HLA-DQ2.5 / Non HLA-DQ8	

Below the Plate layout section is the Main results section, showing the individual allele scores per sample as well as the Primary and Secondary results.



To export your results click on the “Export” button in the upper right corner and choose your file type.

5.3.6 Interpretation of results

Primary

Result	Interpretation
Invalid	The results could not be determined due to lack of proper signal
Undetermined / No Match	The results could not be determined due to unknown genotypes or allele combinations
HLA-DQ2.5	The sample is positive for HLA-DQ2.5
HLA-DQ8	The sample is positive for HLA-DQ8
HLA-DQ2.5 / HLA-DQ8	The sample is positive for HLA-DQ2.5 as well as HLA-DQ8
Non HLA-DQ2.5 / Non HLA-DQ8	The sample is negative for HLA-DQ2.5 as well as HLA-DQ8

Secundary

Result	Interpretation
HLA-DQ2.2	The sample is positive for HLA-DQ2.2
Carrier of the HLA-DQB1*02 allele (Non DQ2.5)	The sample is negative for HLA-DQ2.5 as well as HLA-DQ8, but carrier of the HLA-DQB1*02 allele
Carrier of the HLA-DQA1*05 allele (Non DQ2.5)	The sample is negative for HLA-DQ2.5 as well as HLA-DQ8, but carrier of the HLA-DQA1*05 allele

5.3.7 Analysing the results manually

To analyse the results without using the online analysis tool you can use the analysis chart below.

	MasterMix 1				MasterMix 2			MasterMix 3	
	Melt Peak 1	Melt Peak 2	Melt Peak 3	Melt Peak 4	Melt Peak 1	Melt Peak 2	Melt Peak 3	Melt Peak 1	Melt Peak 2
	HLA-DQA1*02 Tm	HLA-DQA1*03 Tm	HLA-DQB1*02 Tm	HLA control peak ** Tm	HLA-DQA1*05 Tm	HLA control peak ** Tm	HLA-DQB1*0302 Tm	HLA DQ2.5 homozygote SNP * Tm	HLA control peak ** Tm
Genotype	77.3°C - 79.3°C	80.4°C - 82.4°C	83.5°C - 85.5°C	88.0°C - 90.0°C	75.2°C - 77.2°C	80.3°C - 82.3°C	84.2°C - 86.2°C	77.6°C - 79.6°C	80.6°C - 82.6°C
HLA-DQ2.5 heterozygous	-	-	+	+	+	+	-	+	+
HLA-DQ2.5 homozygous	-	-	+	+	+	+	-	-	+
HLA-DQ2.2	+	-	+	+	-	+	-	+	+
HLA-DQ8	-	+	-	+	-	+	+	+	+

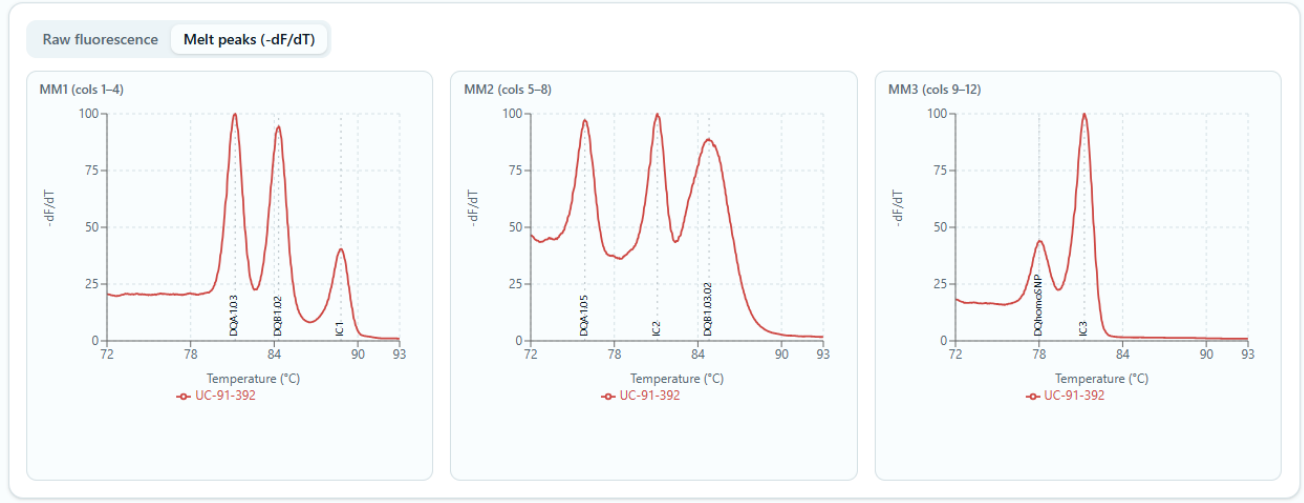
All other melting peak combinations result in non HLA-DQ2.5 / non HLA-DQ8.
If HLA-DQA1*02, HLA-DQA1*03 and HLA-DQA1*05 are all positive it means that the sample is contaminated.

Notes:
* The abscence of the HLA-DQ2.5 homozygote SNP indicates homozygosity for HLA-DQ2.5.
* In case of HLA-DQ2.5 homozygosity no other alleles than HLA-DQA1*05 and HLA-DQB1*02 and the control peaks can be positive.
** The HLA control peak of each reaction should always be positive, indicating the prescence of human DNA in the sample.
*** Positive control MM1 (PC1), should always be positive for HLA-DQA1*02, HLA-DQA1*03, HLA-DQB1*02 and the HLA control in MasterMix 1.
**** Positive control MM2 (PC2), should always be positive for HLA-DQA1*05, HLA-DQB1*0302 and the HLA-control in MasterMix 2.
***** Positive control MM3(PC3), should always be positive for the HLA-DQ2.5 homozygous SNP and the HLA-control in MasterMix 3.

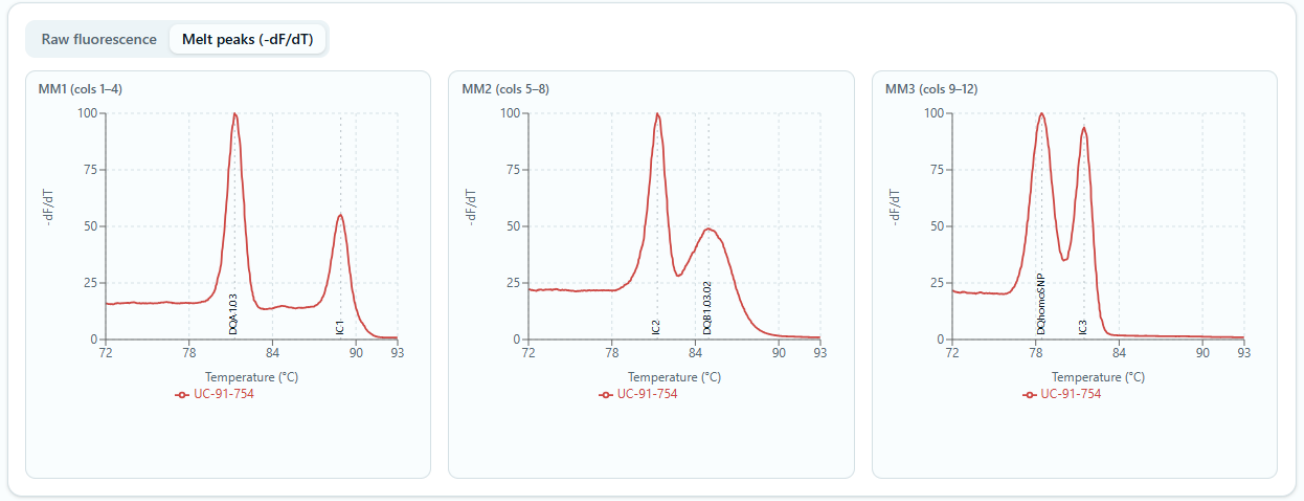
5.3.8 Examples

Below are a few examples of how to interpret the melting curves manually.

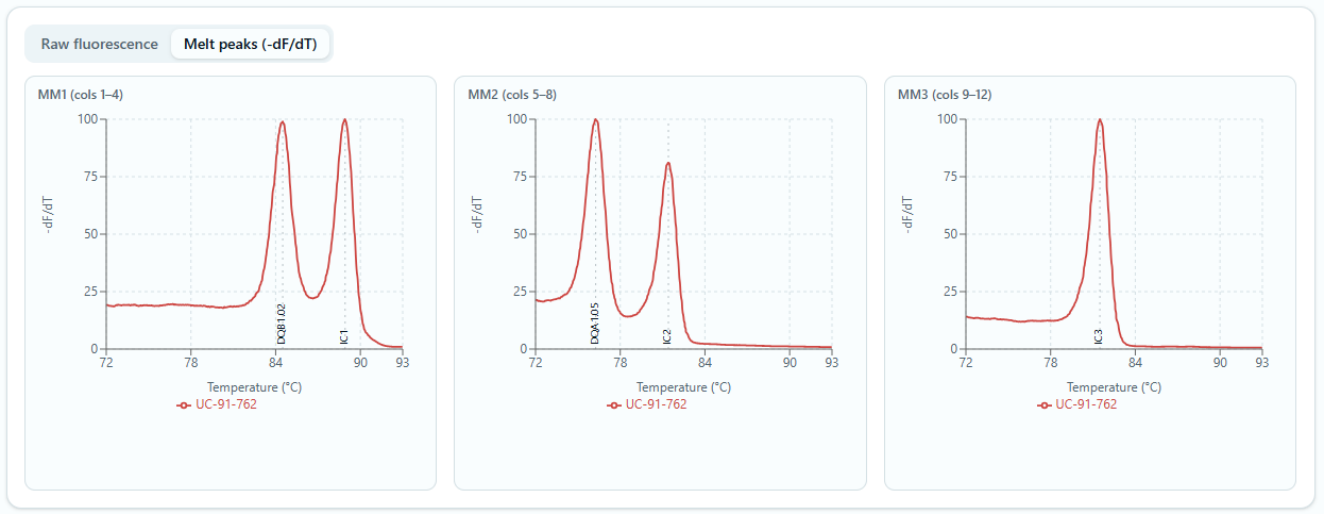
Example 1: HLA-DQ2.5 / HLA-DQ8



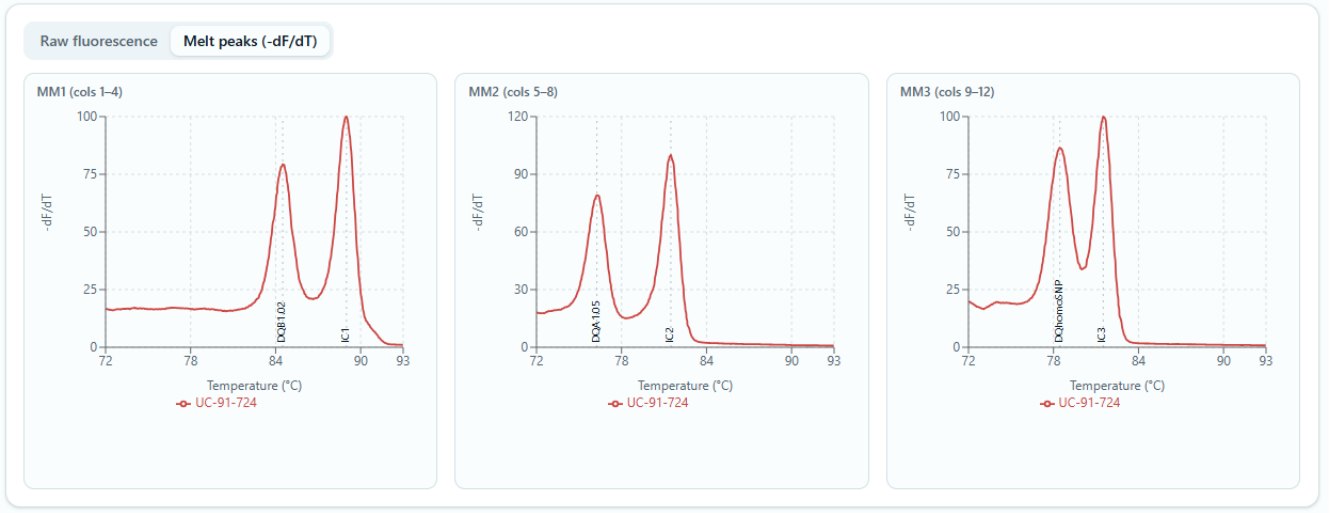
Example 2: HLA-DQ8



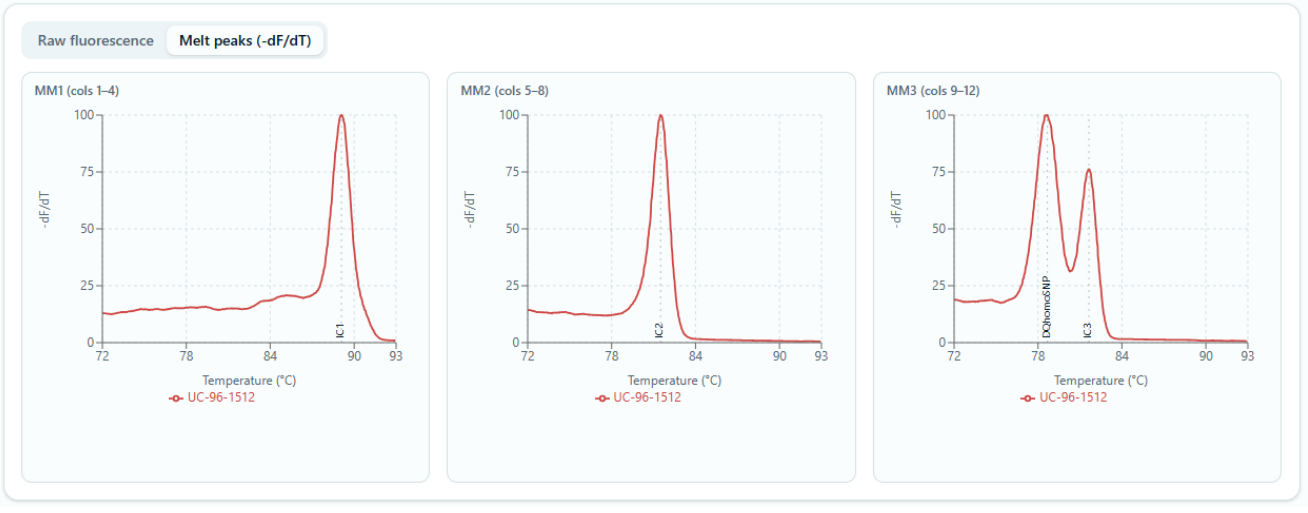
Example 3: HLA-DQ2.5 homozygous



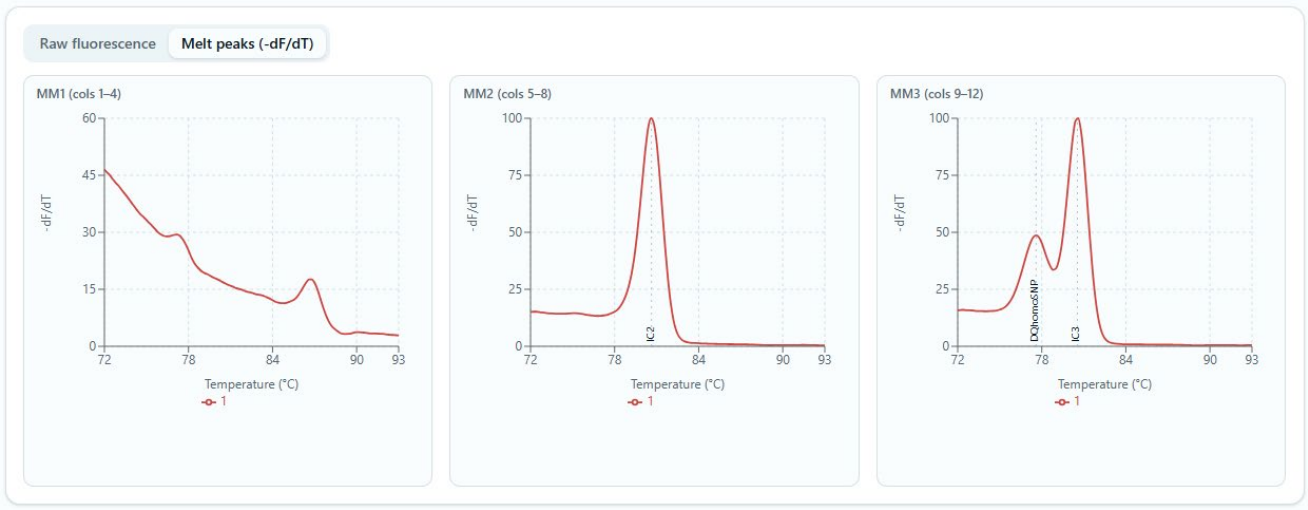
Example 4: HLA-DQ2.5 heterozygous



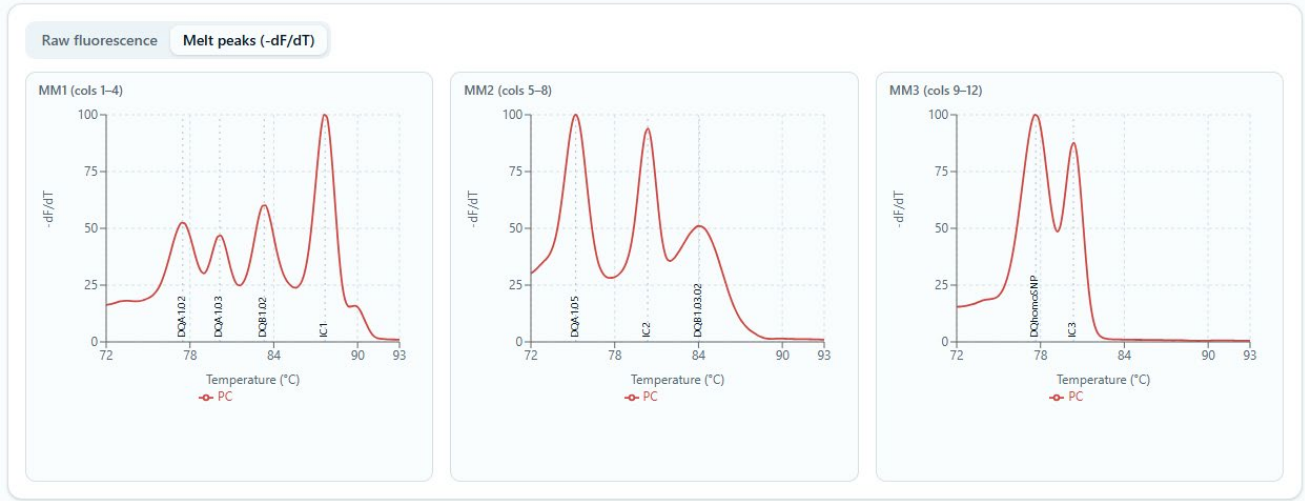
Example 5: Non HLA-DQ2.5 / Non HLA-DQ8



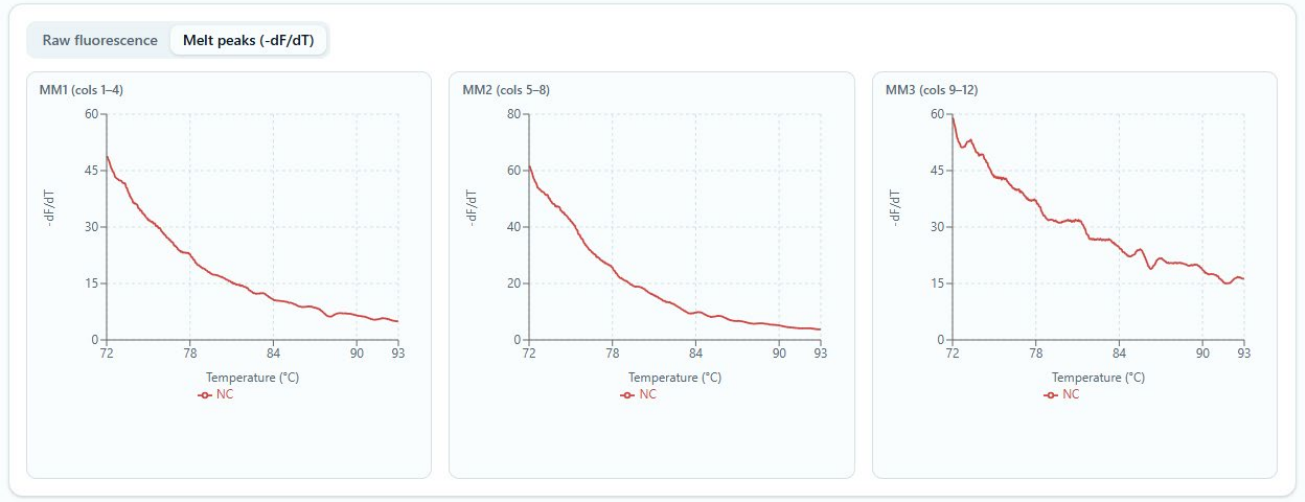
Example 6: Invalid



Example 7: Undetermined / No Match



Example 8: Negative, indicating there is no DNA present in the sample



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).
11. In case any serious incident has occurred in relation to the CeliaSCAN kit, the incident must be reported to Microbe&Lab as well as the competent authorities of the Member State in which the user of this product is established. Contact details for Microbe&Lab can be found at page 23 of this document.

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, the Applied Biosystems® 7500 Real-Time PCR series and the Biorad CFX96 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.
- CeliaSCAN is not intended to diagnose Celiac Disease.

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.

8.0 Version History

Revision	Date	Changes
8.0	October 2023	Added instructions for manual data interpretation
8.1	July 2026	Added instructions for Online Analysis tool Updated instructions for manual interpretation
8.2	July	Updated the interpretation section

List of symbols as used in labeling

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

In vitro diagnostic medical device

Manufacturer

Batch code (Lot)

Use by date

Temperature Limitation

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

WARRANTY

This product is warranted to perform as described in its labeling and in the Microbe&Lab b.v. Literature when used in accordance with all instructions. Microbe&Lab b.v DISCLAIMS ANY IMPLIED WARRANTY OF SPACE MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE and in no event shall Microbe&Lab b.v be liable for consequential damages, Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS TO USE IT SOLELY FOR PROVIDING HUMAN IN VITRO DIAGNOSTIC SERVICES. NO LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

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Science Park 408
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The Netherlands
Tel: +31 (6) 29283265
info@microbenlab.com
KvK: 82161933

For additional information, please visit www.celiascan.eu

Annex 1: Material Safety Data Sheet

1 Identification of the substance/preparation and company

1.1 Product identification

Product name:	CeliaSCAN
Product code:	2331633
Chemical Family:	NA
CAS Number:	NA
Synonyms:	NA

1.2 Manufacturer identification

Microbe&Lab B.V.
Paalbergweg 2-4
1105 AG Amsterdam
The Netherlands

2 Composition/information

Kit Component	**Cat. Number**
Mastermix 1	CS_MM1
Mastermix 2	CS_MM2
Mastermix 3	CS_MM3
Positive control for Mastermix 1	CS_PC1
Positive control for Mastermix 2	CS_PC2
Positive control for Mastermix 3	CS_PC3
Negative control	CS_NC

3 Hazard identification

Normal use:
Health effects (up)on:

Testing -

Inhalation:	Not expected
Eye:	Not expected
Skin:	Not expected
Ingestion:	Not expected
Carcinogenicity:	Not expected
Chronic effects:	Not expected

4 First aid measures

Inhalation:	not known (very unlikely)
Skin contact:	Wash carefully
Eye contact:	Wash carefully
Ingestion:	None

5 Fire-fighting measures

Unusual fire or explosion hazards:	Not expected
Hazardous combustion products:	Not expected

6 Accidental release measures

Spill/ Leak Procedures:	Wear gloves

7 Handling and storage

Handling precautions:	General hygiene measures for the handling of this product are applicable
Storage requirements:	-20 °C

8 Exposure control / personal protection

Ventilation:	None
Personal Protective Equipment:	No special measures necessary

9 Physical and chemical properties

Physical state:	Frozen
Appearance:	NA

10 Stability and reactivity

Conditions to avoid:	None from safety perspective
Hazardous Decomposition Product:	Not expected

11 Toxicological information

Toxicity data:	None
Eye Effects:	Not known
Skin effects:	Not known
Acute Inhalation effects:	Not known/Not expected
Acute Oral effects:	Not known/Not expected
Chronic Effects:	Not known/Not expected
Carcinogenic effects:	Not known/Not expected
Mutagenicity:	Not known/Not expected

12 Ecological information

Do not discharge product unmonitored into environment.

Ecotoxicity:	Not known
Environmental fate:	Not known
Environmental degradation:	Not known
Soil Absorption/ Mobility:	Not Known

13 Disposal considerations

Disposal in agreement with local waste disposal authority. Follow all Regional and National regulations.

14 Transport Information

No hazardous goods

Temperature:	-20 °C or dry ice

15 References

N.A.

16 Other information

This information is based on our present state of knowledge. It should therefore not be construed as guaranteeing specific properties of the products described or their suitability for a particular application.

Approved By	Function	Signature and date
J. Ramnarain	Manager QA/RA