GenVinSet

HLA CELIAC

Instructions for use

Kit for detection of alleles associated to Celiac Disease

For In Vitro Diagnostic Use

Reference GVS-DQ-48 (48 test)
GVS-DQ-24 (24 test)

Store from –18 to –30ºC

CE

Rev 12 / 23-11-2020

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Intended use

GENVINSET® HLA CELIAC is a kit designed for the detection of the HLA-DQB1*02, DQB1*03:02 and DQA1*05 alleles, and the consequent determination of the DQ2 and DQ8 antigens, associated with celiac disease. The kit is capable of determining the homozygous or heterozygous presence of the DQB1*02 allele.

The analysis is based on the Real-Time PCR technology, using TaqMan® probes.

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Summary and explanation

Celiac disease is a malabsorption disorder caused by interactions between genetic and environmental factors. An environmental factor that precipitates the disease development is gluten or related proteins present in cereals such as wheat, barley and rye. Immune intolerance causes a chronic inflammatory response in the mucosa of the small intestine with symptoms such as diarrhea, steatorrhea and weight loss [1, 2].

It is one of the most common diseases in the Caucasian population, with a prevalence of between 1:100 and 1:500 in Europe and North America [3].

Susceptibility to gluten sensitivity is, in part, genetically determined. The strong predisposition is associated with the HLA-DQ alleles, encoding the α and ß chains of two molecules of the Class II Major Histocompatibility Complex (MHC) [2-4].

In most of the studied populations, 90-95% of patients carry the HLA-DQ2 heterodimer encoded by the DQA1*05 and DQB1*02 alleles in *cis* position (more common in central and northern Europe) or in *trans* position (more common in Mediterranean countries). The risk of an individual carrying the DQA1*05 and DQB1*02 heterodimer to suffer from celiac disease is 50 times greater than the average population risk, but its presence does not predict the development of the disease, since it is also present in a quarter of the general healthy population [5, 9, 11-13].

The remaining patients (5-10%) usually carry a second heterodimer, the HLA-DQ8 (more common among South American indigenous patients), encoded by the DQA1*03 and DQB1*03:02 alleles,, or are carriers of one of the alleles that code for the HLA-DQ2 heterodimer separately (DQA1*05 or DQB1*02) [5, 9, 11-13].

The following table shows the HLA haplotypes which constitute the HLA-DQ2 and HLA-DQ8 antigens (specifying the DQB1 and DQA1 alleles and the associated DRB1 allele:

HLA-DQ	Serological equivalent	Genotype						Frequency in celiac patients
		Haplotype 1			Haplotype 2			
		DQB1*	DQA1*	DRB1*	DQB1*	DQA1*	DRB1*	
DQ2	DQ2.5 *cis* heterozygous	02:01	05:01	03	-	-	-	More than 90% of celiac patients
	DQ2.5 *cis* homozygous	02:01	05:01	03	02:01	05:01	03	
	DQ2.5 *cis* + DQ2·2	02:01	05:01	03	02:02	02:01	07	
	DQ2.5 *trans*	03:01	05:05	(11)	02:02	02:01	07	
	DQX.5	03:01	05:05	(11)	-	-	-	
	DQ2.2	02:02	02:01	07	-	-	-	
DQ8	DQ8	03:02	03:01	(04)	-	-	-	2-10% of patients

Table 1. Based on references 3 and 4.

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Figure 1 displays the different DQ heterodimers (composed by their respective alleles) which confer predisposition to celiac disease.

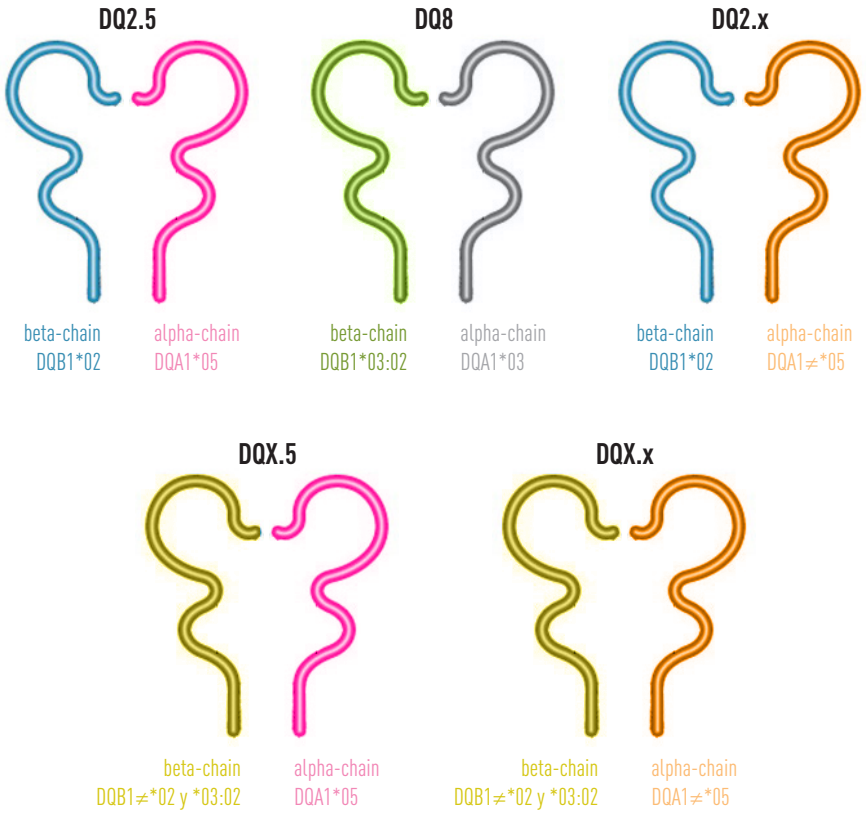

Figure 1 displays the different DQ heterodimers (composed by their respective alleles) which confer predisposition to celiac disease.

The prevalence of celiac disease has been underestimated. Early diagnosis is important to start as soon as possible with a gluten-free diet. The absence of treatment often causes other autoimmune disorders such as type 1 diabetes or rheumatoid arthritis.

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Procedure principles

The detection method used by Genvinset® is based on the Real-Time PCR technology, using TaqMan® probes.

The Genvinset® HLA CELIAC kit is composed by three PCR reactions with specific primers for the HLA-DQB1*02, DQB1*03:02 y DQA1*05 (*) alleles.

The detection of the alleles which confer genetic predisposition to celiac disease allows for the determination of the haplotypes.

Moreover, the method allows the amplification and detection of a control gene (B-Globin) in reactions 2 and 3 (not needed in Reaction 1), which allows to verify the results of the assay.

(*) See "Limitations of the Procedure" (page 23).

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Kit contents

Reference GVS-DQ-48 (48 tests)

- GVS-DQ-PM1: 2 vials x 110 µL Primer Mix 1. (PM1)
- GVS-DQ-PM2: 2 vials x 110 µL Primer Mix 2. (PM2)
- GVS-DQ-PM3: 2 vials x 110 µL Primer Mix 3. (PM3)
- GVS-DQ-MM1: 2 vials x 156 µL Master Mix 1 (MM1)
- GVS-DQ-MM2: 2 vials x 156 µL Master Mix 2 (MM2)
- GVS-DQ-MM3: 2 vials x 156 µL Master Mix 3 (MM3)
- GVS-DQ-C+: 1 vial x 15 µL Positive Control (C+)
- GVS-RB: 1 vial x 100 µL Reaction Blank. (RB)

Reference GVS-DQ-24 (24 tests)

- GVS-DQ-PM1: 1 vial x 110 µL Primer Mix 1. (PM1)
- GVS-DQ-PM2: 1 vial x 110 µL Primer Mix 2. (PM2)
- GVS-DQ-PM3: 1 vial x 110 µL Primer Mix 3. (PM3)
- GVS-DQ-MM1: 1 vial x 156 µL Master Mix 1 (MM1)
- GVS-DQ-MM2: 1 vial x 156 µL Master Mix 2 (MM2)
- GVS-DQ-MM3: 1 vial x 156 µL Master Mix 3 (MM3)
- GVS-DQ-C+: 1 vial x 15 µL Positive Control (C+)
- GVS-RB: 1 vial x 100 µL Reaction Blank. (RB)

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Kit storage

All reagents of the kit should be stored from -18ºC to -30ºC, being stable at this temperature until the expiration date indicated on the label of each vial. Do not perform more than 3 freeze/thawing cycles to the Primer Mix vials (GVS-DQ-PM) as this could reduce the sensitivity of the assay and impair results.

Due to the reagent's photo sensitivity nature, avoid continuous exposure to light.

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Materials required but not supplied

General

- Gloves
- Lab coat

Consumables

- Filter tips (P200, P100 and P10)
- 1.5 mL autoclaved tubes
- Specific consumables for each q-PCR instrument (in case of using Rotor Gene Q, use only 0.1 mL tubes)

Equipment

- q-PCR instruments with detection channels for FAM and HEX. The following instruments have been validated:
  - StepOne™, Applied Biosystems™
  - 7500 Real-Time PCR System, Applied Biosystems™
  - LightCycler® 96 System, Roche
  - LightCycler® 480, Roche
  - Rotor-Gene® Q, Qiagen®
  - CFX96, BioRad
- Vortex mixer
- Pipettes (P200, P100 and P10)

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Sample collection and preparation

This test should only be performed with complete blood samples treated with EDTA anti coagulation agents or citrate. Heparin can interfere with the PCR process and should not be used in this procedure.

The technique is compatible with any conventional DNA extraction system. Before using the obtained results for diagnostic purposes, it is highly recommended to evaluate the DNA extraction method with the Genvinset® HLA CELIAC kit.

Caution

All biological and blood samples should be treated as potentially infectious. When manipulating them, follow all basic (universal) precautions. All sample manipulation should be done wearing gloves and all the appropriate personal protection.

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Usage procedures

A) PCR preparation

Precautions

- Thaw all of the kits components before starting the assay, mix and centrifuge.
- Work on ice or over a cool block.
- The setup of the PCR should be done in the Pre-PCR area.
- Use only filtered tips and 1.5 mL autoclaved tubes.
- Always wear gloves and a lab coat.
- In each session it is recommended to test the contamination control included in the kit (Reaction Blank), and the Positive Control provided with the kit (GVS-DQ-C+) whose typing is DQB1*02/03:02 and DQA1*05.

1. Take the samples out of the freezer. Vortex (or tap it with the finger).

2. Prepare a mix using each one of the Primer Mixes (PM1, PM2 and PM3) with the corresponding Master Mix (MM1, MM2 and MM3), calculating volumes for n+1 samples:

	Vol. per sample (µL)
Master Mix 1, 2 or 3	5
Primer Mix 1, 2 or 3	4

3. Pipette 9 µL of these mixes in the PCR wells. Then, add 1 µL of the DNA sample, positive control or Reaction Blank.

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4. Put the cap on the wells or seal the plate and briefly centrifuge them/it to ensure that all the volume is placed at the bottom of the well.

5. Place the wells/plate in the thermal cycler and start the program.

B) Thermal cycler configuration

1. Set up the following amplification program:

	Cycle Number	Temperature (ºC)	Time (mm:ss)	Ramp (%)	Analysis
Denaturalization	1	95	05:00	100	X
Cycles	40	95	00:15	100	X
		64	1:00	100	Single
Cooling	1	15	∞	100	X

2. Set up the reading channels.

The emitted fluorescence must be read in FAM (495-520 nm) and HEX (535-554 nm) channels. Both fluorescences should be detected in every well (biplex reaction).

NOTE – Special settings for Rotor Gene Q:

a. Open the Rotor-Gene Q – Pure Detection software. Within the "New Run" window, choose the "Advanced" tab and click on "New".

b. Select the type of rotor (only 0.1 mL tubes accepted, see section 'Materials required but not suplied', page 8). Check the "Locking Ring Attached" box and then click on "Next".

c. Set the "Reaction Volume" at 10 µL. Then, insert the name of the operator and the samples.

d. Click on "Edit Profile" and set up the amplification program (see subsection 'B) Thermal Cycler Configuration'). For the step 60 sec at 64ºC select the option "Acquiring to Cycling A". Select the "Green" and "Yellow" channels for fluorescence acquisition. Finally, click on "OK" to accept and close the "Edit Profile" window.

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e. Within the "Run New Wizard" dialog box, click on "Gain Optimisation" to open the "Auto-Gain Optimization Setrup" window. In the "Channel Settings" scrolling menu choose "Acquiring Channels" and then click upon "Add".. In the window "Auto-Gain Optimisation Channel Settings", set the following parameters for each channel ("Green" and "Yellow"):

- Tube position = 1
- Target Sample Range: 5 FI up to 10 FI
- Acceptable Gain Range: -10 to 10

6. Check the option "Perform Optimisation Before 1st Acquisition", and click on "Close".

7. Finally, in the "New Run Wizard" window, clcik on "Next" and "Start Run".

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Results

The GENVINSET® HLA CELIAC kit constitutes a qualitative technique for the determination of the HLA-DQB1*02, DQB1*03:02 and DQA1*05 alleles.

The presence of any of the alleles associated with celiac disease will be determined by the positivity or negativity of the three test reactions (presence or absence of HLA-DQB1*02, DQB1*03:02 and DQA1*05 alleles). In the case of the DQB1*02 allele, the detection of fluorescence in the FAM channel will indicate the presence of, at least one DQB1*02 allele, whereas the detection of fluorescence in the HEX channel will indicate the presence of, at least, one allele different than the DQB1*02.

Each sample will be tested in three wells, one for every reaction (1, 2 and 3). Set the following 5 acquiring channels:

Target Name	Reporter
B-GLOBIN	HEX / VIC
DQB1*02	FAM
No DQB1*02	HEX/VIC
DQA1*05	FAM
DQB1*03:02	FAM

It is not necessary to set any passive reference.

Each reaction, two reading channels have to be set (as indicated above). In Reaction 1, samples generating only an amplification curve in the FAM channel are considered DQB1*02 homozygous. They are identified by a numeric value defined as Crossing Point (Cp), which corresponds to the cycle in which the amplification starts and is detected. On the other hand, when a sample reports an amplification curve only in the HEX channel is is to be considered negative for DQB1*02. If a sample reports amplification curves in both channels, it is to be considered heterozygous for DQB1*02 (it is composed by an DQB1*02 allele and another allele other than DQB1*02).

For Reaction 2, samples generating an amplification curve in the FAM channel are to be considered as DQA1*05 positives. Similarly, samples reporting an amplification curve in the FAM channel of reaction 3 are to be considered as DQB1*03:02 positive.

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The ß-Globin gene is used as a control gene, therefore, it must be detected in all DNA samples in Reactions 2 and 3. Thus, only those DNA samples that report an amplification curve in the HEX/VIC channel will be valid. The criteria specified in the "Quality Control" (page 17) section must be followed at all times.

Figures 2 to 6 show examples of expected results for the different sample types:

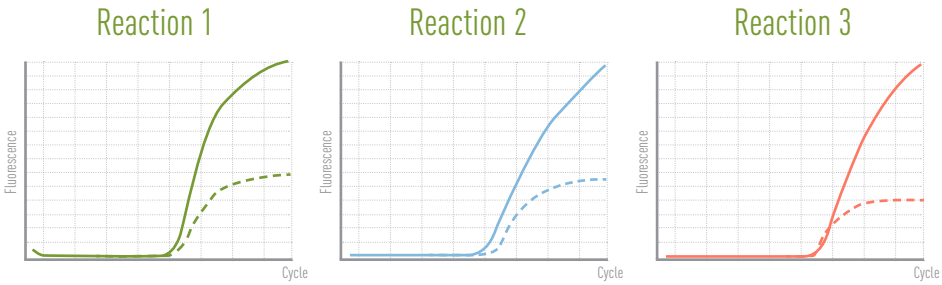

Figure 2. DQ2.5 heterozygous + DQ8.

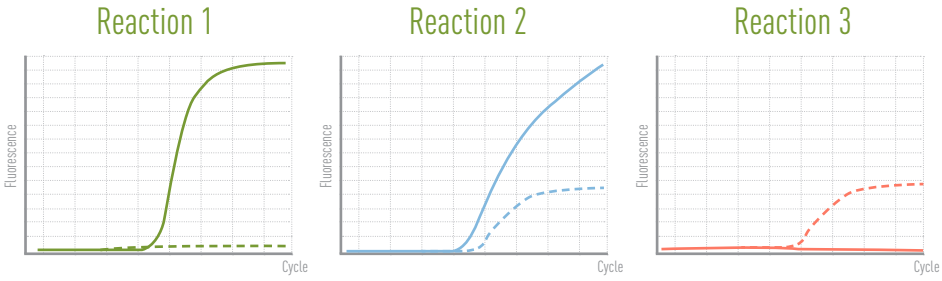

Figure 3. DQ2.5 homozygous

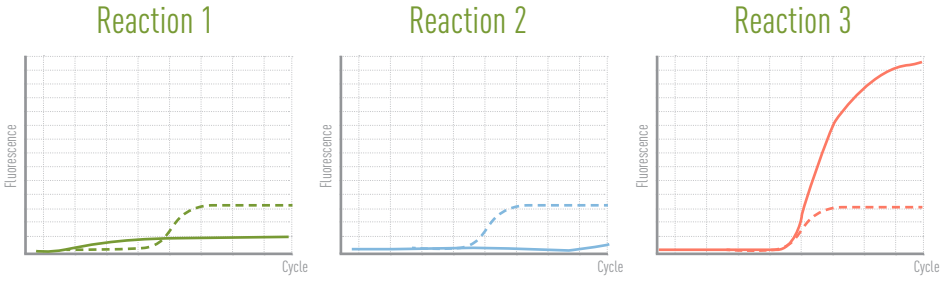

*Figure 4. DQB1*03:02 positive*

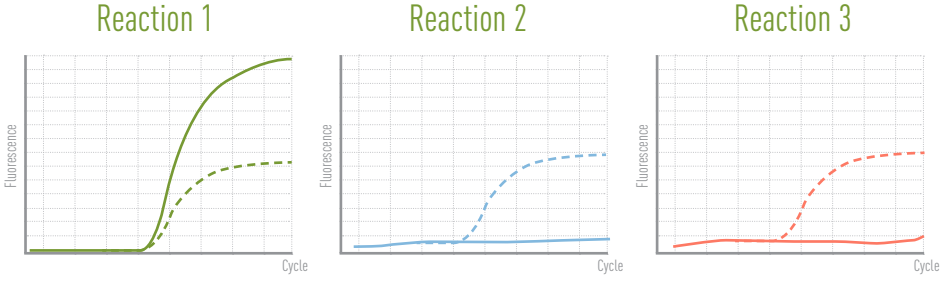

Figure 5. DQ2.x heterozygous

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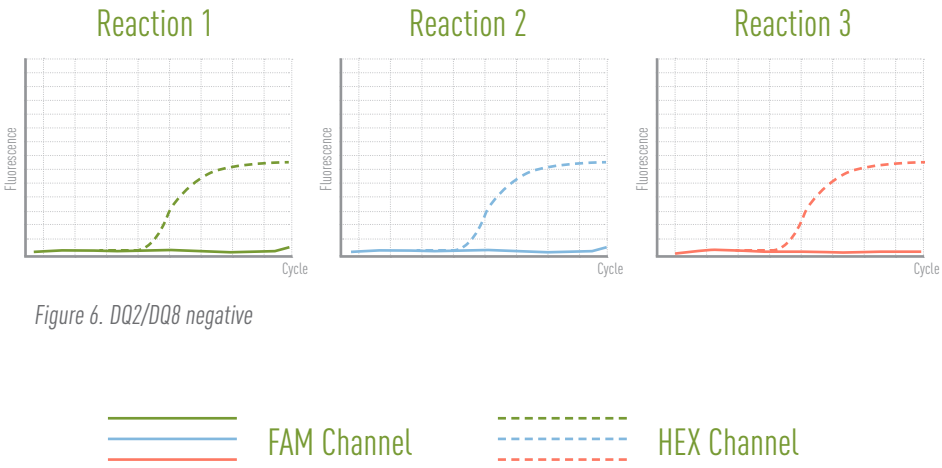

Figure 6. DQ2/DQ8 negative

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Quality control

Due to the qualitative nature of this test, it will not be necessary to perform a calibration.

It is recommended to use the Reaction Blank supplied with the kit as a contamination control. Also, it is advisable to include the Positive Control (DQB1*02/03:02 and DQA1*05 positive) in each assay.

The assay should comply with the following criteria for it to be considered as valid:

- The contamination control (Reaction Blank) must report negative results for all reactions. When the Reaction Blank reports a Cp>35 value it is to be considered as negative whereas a Cp<35 indicated a possible contamination. In that case, the session has to be considered invalid.
- The Positive Control sample must provide positive results for all DQB1*02 / DQB1*03:02 / DQA1*05 and B-Globin genes.
- DNA samples should always be positive for ß-globin (Cp<35).
- DNA samples generating results with Cp>35 for ß-globin and/or DQB1*02 / DQB1*03:02 / DQA1*05 loci must be considered as doubtful and must be retested performing a new extraction of DNA.

The assay must be carried out according to the kits recommendations, as well as other quality control procedures that comply with local, federal and/or certifying agencies specifications.

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Specific operation data

1. Analytical specificity

The alignment of primers and probes in the most common HLA database (IMGT-HLA) has revealed the absence of non-specific bindings. No cross-reaction phenomena with genomic DNA have been reported.

The specificity of the analysis reactions is detailed in section 'Alleles detected by GENVINSET® HLA CELIAC', page 20.

2. Analytical sensitivity

Once performed a dilution assay using 1:4 serialized dilutions of several DNA samples with positive and negative DQB1*02/03:02 and DQA1*05 typings, obtained by conventional extraction system, the following analytitical sensitivity results were obtained:

- Reaction 1 – Detección DQB1*02: Detection Limit = 0,8 ng/uL
- Reaction 2 – Detección DQA1*05: Detection Limit = 0,8 ng/uL
- Reaction 3 – Detección DQB1*03:02: Detection Limit = 2,5 ng/uL

DNA sample obtained by conventional extraction system:
Detection Limit = 2,5 ng/µL (*)

(*) Cp < 35

3. Diagnostic sensitivity and specificity

In several studies of human genomic DNA, 153 samples were tested with Reactions 1, 2 and 3. These samples were previously typed for HLA-DQB1 and DQA1 loci, by SSO (Sequence Specific Oligonucleotides probes) or NGS (Next-Generation Sequencing).

All samples could be validated (positive amplification of the ß-globin control gene). A total of 11 samples were called as homozygous for DQB1*02, 65 were heterozygous and 77 resulted negative for this allele. 66 samples analyzed for Reaction 2 were positive for DQA1*05, and 31 of the samples analyzed for DQB1*03:02 resulted as positive.

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		Reaction 1 - DQB1*02		
	Samples	Homocygous	Heterocygous	Negative
SSO	Homozygous	11	0	0
	Heterocygous	0	65	0
	Negative	0	0	77

		Reaction 2 - DAQ1*05		Reaction 3 - DQB1*03:02	
	Samples	Pos.	Neg.	Pos.	Neg.
SSO	Pos.	66	0	31	0
	Neg.	0	87	0	122

There is a 100% match in the results obtained with GENVINSET® HLA CELIAC and previous information of samples typed by SSO or NGS methodologies.

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Alleles detected by GENVINSET® HLA CELIAC (IMGT-HLA 3.37.0)

Reaction 1

DQB1*02:01:01

DQB1*02:02:01:01

DQB1*02:02:01:02

DQB1*02:02:01:03

DQB1*02:02:01:04

DQB1*02:02:04

DQB1*02:02:06

DQB1*02:109

DQB1*02:143

DQB1*02:144

DQB1*02:156

DQB1*02:53Q

DQB1*02:62

DQB1*02:79

DQB1*02:80

DQB1*02:81

DQB1*02:82

DQB1*02:83

DQB1*02:84

DQB1*02:96N

Reaction 2

DQA1*05:01:01:01

DQA1*05:01:01:02

DQA1*05:01:01:03

DQA1*05:01:01:04

DQA1*05:01:04

DQA1*05:01:05

DQA1*05:01:06

DQA1*05:03:01:01

DQA1*05:03:01:02

DQA1*05:03:02

DQA1*05:05:01:01

DQA1*05:05:01:02

DQA1*05:05:01:03

DQA1*05:05:01:04

DQA1*05:05:01:05

DQA1*05:05:01:06

DQA1*05:05:01:07

DQA1*05:05:01:08

DQA1*05:05:01:09

DQA1*05:05:01:10

DQA1*05:05:01:11

DQA1*05:05:01:12

DQA1*05:05:01:13

DQA1*05:05:01:14

DQA1*05:05:01:15

DQA1*05:05:01:16

DQA1*05:05:01:17

DQA1*05:05:01:18

DQA1*05:05:01:19

DQA1*05:05:01:20

DQA1*05:05:01:21

DQA1*05:05:02

DQA1*05:06:01:01

DQA1*05:06:01:02

DQA1*05:07

DQA1*05:08

DQA1*05:09

DQA1*05:10

DQA1*05:11

DQA1*05:12

DQA1*05:13

DQA1*05:14

DQA1*05:15N

DQA1*05:16

DQA1*05:17N

DQA1*05:18

DQA1*05:19

DQA1*05:19

- Detected Allele
- Non detected Allele
- Non tested allele. Possible weak amplification.
- ***CWD alleles marked in bold and italics***

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Reaction 3

DQB1*03:02:01:01	DQB1*03:02:21	DQB1*03:190
DQB1*03:02:01:02	DQB1*03:02:22	DQB1*03:199
DQB1*03:02:01:03	DQB1*03:02:23	DQB1*03:203
DQB1*03:02:01:04	DQB1*03:02:24	DQB1*03:204
DQB1*03:02:01:05	DQB1*03:02:25	DQB1*03:205
DQB1*03:02:01:06	DQB1*03:02:26	DQB1*03:210
DQB1*03:02:01:07	DQB1*03:02:27	DQB1*03:211
DQB1*03:02:01:08	DQB1*03:02:28	DQB1*03:213N
DQB1*03:02:02	DQB1*03:02:29	DQB1*03:214
DQB1*03:02:03	DQB1*03:02:30	DQB1*03:215
DQB1*03:02:04	DQB1*03:07	DQB1*03:223
DQB1*03:02:05	DQB1*03:08	DQB1*03:224
DQB1*03:02:06	DQB1*03:106	DQB1*03:225
DQB1*03:02:07	DQB1*03:107	DQB1*03:228
DQB1*03:02:08	DQB1*03:11	DQB1*03:229
DQB1*03:02:09	DQB1*03:125	DQB1*03:233
DQB1*03:02:10	DQB1*03:136	DQB1*03:237N
DQB1*03:02:11	DQB1*03:141	DQB1*03:240
DQB1*03:02:12	DQB1*03:146	DQB1*03:245
DQB1*03:02:13	DQB1*03:153	DQB1*03:247
DQB1*03:02:14	DQB1*03:161	DQB1*03:251
DQB1*03:02:15	DQB1*03:174	DQB1*03:261
DQB1*03:02:16	DQB1*03:175	DQB1*03:263
DQB1*03:02:17	DQB1*03:178	DQB1*03:265
DQB1*03:02:18	DQB1*03:179	DQB1*03:273
DQB1*03:02:19	DQB1*03:18	DQB1*03:274
DQB1*03:02:20	DQB1*03:189	DQB1*03:277

- Detected Allele
- Non detected Allele
- Non tested allele. Possible weak amplification.
- ***CWD alleles marked in bold and italics***

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Alleles detected by GENVINSET® HLA CELIAC (IMGT-HLA 3.37.0)

DQB1*03:278
DQB1*03:279
DQB1*03:287
DQB1*03:295
DQB1*03:296
DQB1*03:298
DQB1*03:299
DQB1*03:300
DQB1*03:301
DQB1*03:308
DQB1*03:310N
DQB1*03:315
DQB1*03:32
DQB1*03:320
DQB1*03:321
DQB1*03:322
DQB1*03:323
DQB1*03:324
DQB1*03:333
DQB1*03:334N
DQB1*03:339N
DQB1*03:343
DQB1*03:344
DQB1*03:345
DQB1*03:348
DQB1*03:349
DQB1*03:352
DQB1*03:362
DQB1*03:364
DQB1*03:367
DQB1*03:368
DQB1*03:369
DQB1*03:37
DQB1*03:371
DQB1*03:379
DQB1*03:383
DQB1*03:386
DQB1*03:388
DQB1*03:392
DQB1*03:45:01
DQB1*03:45:02
DQB1*03:62
DQB1*03:63
DQB1*03:64
DQB1*03:66N
DQB1*03:67
DQB1*03:68
DQB1*03:70
DQB1*03:81
DQB1*03:85

- Detected Allele
- Non detected Allele
- Non tested allele. Possible weak amplification.
- ***CWD alleles marked in bold and italics***

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Limitations of the procedure

- The PCR should be carried out in the precise conditions described in this procedure. Any deviations could lead to poor results.
- Any mutations or polymorphisms happening at the annealing primer/probes sites can prevent the amplification and cause the lack of allele definition. In this case, other technologies could be required to resolve the typing.
- All GENVINSET® work must be done according to general lab best practices and local regulations, such a the EFI standard (European Federation of Immunogenetics).
- The qPCR thermal cycler must be calibrated according to the manufacturer's recommendations and should be used in complying with the instructions.
- Do not mix components from other kits or lot numbers.
- Do not use the kit beyond its expiration date.
- Do not use the kit if there are any suspicions of possible loss of reactivity, contamination, container deterioration or any other incidence that might affect the kits performance.
- Due to the complexity of the HLA typing, data and results interpretation should be revised by qualified personnel.
- Discard any expired reagents accordingly to the applicable regulations.

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Troubleshooting guide

Problem

- **Probable cause(s)**
  - Suggested corrective measure(s).

Contamination control (RB) is positive is positive

- **Primer Mix / Master Mix / Reaction Blank**
  - Repeat the experiment with new Primer Mix / Master Mix / Reaction Blank aliquots.
  - Always manipule the kits components accordingly to the accepted lab practices in order to avoid any contamination.
  - Make sure that the kit has been used and sotred following the indications of this protocol.
  - Discard any contaminated reagents.
- **Pre-PCR area is contaminated**
  - Confirm that all necessary precautions in the PCR area have been followed.
  - Check for possible contamination problems in other PCR techniques.
  - Check the suitability of the consumables used (1.5 mL tubes, pipette tips).
  - Confirm there is no Taq contamination.
- **Pipetting error**
  - Check that the added sample corresponds to the one assigned in the worksheet.

Low or no signal in all samples. Control samples are OK

- **Bad quality of DNA samples**
  - Repeat the DNA extraction.
- **Samples with very low DNA concentration**
  - Check the DNA concentration.

Fluorescence intensity too low

- **Kit degradation (Primer Mix vial)**
  - Check that the kit has been properly stored, following the indications specified in this protocol.
  - Avoid performing more than 3 freeze/thawing cycles to the Primer Mix vial.

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- Aliquote the reagents if necessary.
- Repeat the assay using a new batch of reagents.

- **Kit degradation (Master Mix vial)**
  - Check that the kit has been properly stored, following the indications specified in this protocol.
  - Avoid performing more than 3 freeze/thawing cycles to the Primer Mix vial.

Negative control sample is positive

- **Cross contamination**
  - Always handle the kits components following all the appropriate contamination avoidance practices.
- **Pipetting error**
  - Check that the added sample corresponds to the one assigned in the worksheet.

Positive control sample is negative

- **Pipetting error**
  - Check that the added sample corresponds to the one assigned in the worksheet.

Fluorescence intensity varies

- **There is dirt in the PCR tube, which interferes with the signal**
  - Always wear gloves when manipulating the PCR tubes/plate.
- **The volume is not at the bottom of the well or there is an air bubble**
  - Centrifuge the PCR tubes/plate to ensure that the volume is placed at the bottom and that all air bubbles are removed.
- **Pipetting error**
  - Check that the volume added in each well is correct.

There is no fluorescence signal

- **Incorrect reading channels selected**
  - Check that the reading channels have been correctly set.
- **Pipetting error or reagent absence**
  - Check for any pipetting errors.
  - Repeat the PCR.
- **No reading channel was selected in the thermal cycler's program.**
  - Check the set up of the thermal cycler program and modify it if necessary.

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Changes to version 10

Version	Description of the modification
Rev. 10	Reduction of number of PCR cycles
Rev. 11	Addition of the CFX96 (BioRad) thermal cycler to the list of validated instruments

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	Expiration date
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	Store at
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	Positive control

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