Genvinset®

HLA Celiac

INSTRUCTIONS FOR USE

Kit for detection of alleles associated to Celiac Disease

For In Vitro diagnostic use

Rev. 14 / 2022-10-20

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www.bdrdiagnostics.com

CE

Reference No.	UDI-DI.	Store
GVS-DQ-24 (24 tests)	8437016942093	from -30°C to -18°C
GVS-DQ-48 (48 tests)	8437016942109	

Genvinset®

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1- Information for safety

Please, read completely these instructions for use and follow them during the use of the current IVD kit.

The IVD kit shall be used by experts with strong experience in DNA analysis and interpretation of genetic results.

Consult the manufacturer if there are doubts involving the description of the assay method. Contact by phone +34 976 094 603 or email address *customersupport@bdrdiagnostics.com*.

The IVD kit has a limited shelf life. Make sure that the shelf life is not expired before using the kit. Reagents from a kit beyond its expiry date might be degraded, which could impair results. Discard the expired reagents following applicable regulations.

The contamination of the sample or the reagent can render incorrect results. Be careful during the DNA extraction and sample and reagents manipulation.

This kit can be damaged during transport or storage. Do not use the kit in case of suspected damage during transport. Follow carefully the storage conditions described in the label and the IFU.

Ensure that the waste is managed according to local regulations. Incorrect waste management can result in environmental contamination.

The toxicological properties of the kit have not been studied in-depth, so it is recommended to avoid contact with skin and mucous membranes. Do not ingest. Manual Safety Data Sheets (MSDS) are available for the customer by request.

Ensure that this kit is adequate for the requested analysis by the clinician.

2- Intended Use

Genvinset® HLA Celiac is a semi-automated in vitro diagnostic kit for the qualitative detection of the HLA-DQB1*02, DQB1*03:02 and DQA1*05 alleles in genomic DNA extracted from whole blood, and the consequent determination of the DQ2 and DQ8 antigens, associated with celiac disease predisposition. The kit is able to determine the homozygosity or heterozygosity status for the DQB1*02 alleles. The analysis is based on Real-Time PCR technology, using TaqMan® probes.

Patients who can benefit from this determination are those referred by a specialist. The results of this test should not be the only ones on which the therapeutic decision is based and should be used as an aid in the diagnosis together with results of other markers of the disease.

The intended user of the kit is technical personnel trained to carry out the protocol and the interpretation of results described in this document.

3- Summary and explanation

Celiac disease (CeD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically predisposed individuals[1].

CeD 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[2], and affecting all age groups,

including the elderly. While CeD is common around the globe and is rising in prevalence in many populations, it is frequently undetected in clinical practice[3]. Currently, the only treatment for celiac disease is a life-long, strict gluten-free diet leading to improvement in quality of life, ameliorating symptoms, and preventing the occurrence of refractory celiac disease, ulcerative jejunoileitis, and small intestinal adenocarcinoma and lymphoma[4].

While environmental factors are important for CeD development, a notable feature of the disease is its high heritability and strong association with two sets of HLA alleles[5]: DQA1*05-DQB1*02 and DQA1*03-DQB1*03:02, which code for class II MHC DQ2 and DQ8 molecules, respectively[6,7,8]. The occurrence of CeD in the absence of these DQ risk factors is extremely rare. The presence of these molecules does not predict the development of CeD, since they are present in 25 to 50% of the general population, so the vast majority of these individuals will never develop the disease[9].

90% of Caucasian CeD patients have the HLA-DQ2.5 haplotype (encoded by the DQA1*05:01 and DQB1*02:01 alleles) either in cis (more common in central and northern Europe) or trans (more common in Mediterranean countries) positions. HLA-DQB1*02:01 is highly associated with increased disease risk; as patients carrying two copies of HLA-DQB1*02:01 show a five-fold risk to develop the disease compared to heterozygotes. The dosage of DQA1*05:01 appears less closely linked to the development of celiac disease[10].

The rest of the patients (5-10%) usually either carry a second heterodimer, HLA-DQ8 (most common among south American indigenous patients), encoded by the DQA1*03:01 and DQB1*03:02 alleles, or carry any of the alleles that code for the HLA-DQ2 heterodimer separately (DQA1*05 or DQB1*02)[11].

The following table shows the HLA haplotypes which constitute the HLA-DQ2 and HLA-DQ8 antigens: the DQB1 and DQA1 alleles encoding the α and β chains of the HLA antigen, and the associated DRB1 allele are displayed[7,8].

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

Less than 1% of CeD patients lack these HLA haplotypes[11] so their absence can assist in the clinical setting to exclude a diagnosis of the disease. Nevertheless, more than 70 candidate genes in over 40 non-HLA loci have been implicated in CeD heritability[12,13,14,15]. The relevance of these additional genes in conferring genetic risk for CeD is rather limited, but they may lead to the discovery of key pathways potentially involved in disease pathogenesis[4].

HLA molecular typing for CeD is, therefore, a genetic test with a high negative predictive value. What is more, it is an important tool able to discriminate individuals genetically susceptible to CeD, especially in at-risk groups such as first-degree relatives (parents, siblings and offspring) of patients and in presence of autoimmune conditions (type 1 diabetes,

thyroiditis, multiple sclerosis) or specific genetic disorders (Down, Turner or Williams syndromes)[16].

4- Procedure principles

The test is based on Real-Time PCR technology with TaqMan® probes. Each sample is analysed in 3 reactions. The following alleles/genes are analysed in each reaction:

Reaction	HLA detected alleles	Control gene
1	DQB1*02 and NO DQB1*02	N/A
2	DQA1*05	*HBB* (β-globin, IPC)
3	DQB1*03:02	*HBB* (β-globin, IPC)

In Reaction **1**, samples are analysed with:

- A pair of primers for the amplification of HLA-DQB1 alleles.
- A hydrolysis probe specific for the HLA-DQB1*02 alleles labelled at the 5` end with FAM fluorophore and a hydrolysis probe specific for the alleles different from HLA-DQB1*02 (non-HLA-DQB1*02) labelled at the 5' end with HEX fluorophore. This reaction allows the determination of the zygosity of the HLA-DQB1*02 alleles.

In Reactions **2** and **3**, samples are analysed with:

- A pair of primers for the amplification of HLA-DQA1*05 and HLA-DQB1*03:02 alleles in Reactions 2 and 3 respectively, and a pair of primers for β-globin (*HBB*) gene amplification that serves as an internal positive control (IPC).
- A hydrolysis probe specific for the analysed HLA alleles (HLA-DQA1*05 and HLA-DQB1*03:02, for Reactions 2 and 3 respectively) labelled at the 5` end with FAM fluorophore and a hydrolysis probe specific for the IPC labelled at the 5' end with HEX fluorophore.

All the probes are labelled at the 3' end with a quencher that inhibits fluorescence emission when the probe is intact.

As the PCR reaction proceeds, the 5'→3'exonuclease activity of Taq polymerase cleaves the probes attached to their complementary sequence, separating the fluorophore from the quencher and producing a fluorescent signal (in Real-Time), which is proportional to the amount of PCR product generated and monitored in a Real-Time PCR instrument. Thus:

In Reaction **1**:

- In the case of homozygous samples for the HLA-DQB1*02 alleles, the specific FAM-labelled probe binds to its complementary DNA sequence and the following is observed:
    - Fluorescent signal in the FAM channel (λmax 518 nm) and
    - No signal or weak signal in the HEX channel (λmax 556 nm).
- In the case of heterozygous samples for the HLA-DQB1*02 alleles (one copy of the HLA-DQB1*02 allele), both FAM and HEX probes shall bind to their complementary DNA sequences generating:
    - FAM channel signal and
    - Signal in the HEX channel.

- In the case of HLA-DQB1*02 negative samples (without any copy of the HLA-DQB1*02 allele), the specific HEX-labelled probe binds to its complementary DNA sequence and the following is observed:
    - No signal or weak signal in the FAM channel and
    - Fluorescent signal in the HEX channel.

In Reactions **2** and **3**:

- In the case of samples with one or two copies of the analysed HLA alleles (HLA-DQA1*05 or HLA-DQB1*03:02 for Reaction 2 and 3, respectively), the specific FAM-labelled probes bind to its complementary DNA sequences, the IPC specific HEX-labelled probe binds to its complementary DNA sequence and the following is observed:
    - Fluorescent signal in the FAM channel (λmax 518 nm) and
    - Fluorescent signal in the HEX channel (λmax 556 nm).
- In the case of samples with no copies of the analysed HLA alleles (HLA-DQA1*05 or HLA-DQB1*03:02 for Reactions 2 and 3, respectively), the IPC-specific HEX-labelled probe binds to its complementary DNA and the following is observed:
    - No signal or weak signal in the FAM channel and
    - Fluorescent signal in the HEX channel.

5- Kit contents

Reference No.	Vial	Colour	GVS- DQ-24 (24 tests)	GVS- DQ-48 (48 tests)
GVS-DQ-PM1	Primer Mix 1 (PM1)	Blue cap	1 x 192 µL	2 x 192 µL
GVS-DQ-PM2	Primer Mix 2 (PM2)	Blue cap	1 x 192 µL	2 x 192 µL
GVS-DQ-PM3	Primer Mix 3 (PM3)	Blue cap	1 x 192 µL	2 x 192 µL
GVS-DQ-MM1	Master Mix 1 (MM1)	Red cap	1 x 240 µL	2 x 240 µL
GVS-DQ-MM2	Master Mix 2 (MM2)	Red cap	1 x 240 µL	2 x 240 µL
GVS-DQ-MM3	Master Mix 3 (MM3)	Red cap	1 x 240 µL	2 x 240 µL
GVS-DQ-C+	Positive Control (C+)	Green cap	1 x 30 µL	1 x 30 µL
GVS-RB	Reaction Blank (RB)	Natural cap	1 x 100 µL	1 x 100 µL

6- Kit storage

All the components of the kit must be stored between -30°C and -18°C upon reception. Under these conditions, they are stable up to their expiry date indicated on the label.

Do not perform more than 3 freeze/thaw cycles to the kit vials as this may reduce the sensitivity of the assay and affect the results. If assays are to be performed with few samples, it is recommended to use aliquots of the reagents to avoid exceeding the recommended number of freeze/thaw cycles.

Due to the photosensitive nature of the Primer Mix, avoid continuous exposure to light.

7- Materials required but not supplied

General

- Disposable gloves
- Lab coat

Consumables

- Filter tips (P200, P20 and P10)
- 1.5 mL autoclaved tubes
- Compatible consumables for each Real-Time PCR instrument

Equipment

- Vortex mixer
- Centrifuge
- Micropipettes (P200, P20 and P10)
- Real-Time PCR instrument, with FAM and HEX/VIC detection channels. The following devices have been validated:
    - 7500, QuantStudio 5 Dx Real-Time PCR Systems, Applied Biosystems™.
    - LightCycler® 96 and LightCycler® 480 systems, Roche.
    - Rotor-Gene® Q, Qiagen®.
    - DT Lite Real-Time PCR System, DNA-Technology.
    - CFX 96 Real-Time PCR, BioRad.
    - Mic qPCR, Bio Molecular Systems.

8- Sample collection and preparation

Samples must be collected in line with the instructions for use of the collection device (not included with the kit) and under national and international guidelines.

The present test should only be performed with DNA extracted from whole blood samples preserved with anticoagulant agents such as EDTA or citrate. Heparin can interfere with the PCR process and should be avoided.

The technique is compatible with several DNA extraction methods. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

CAUTION!

All biological and blood samples should be treated as potentially infectious. When manipulating them, take the corresponding basic and universal precautions.

9- Usage procedures

➔ PCR setup

CAUTION!

- Define pre- and post-PCR work areas that should be kept separated to reduce the risk of contamination. Prepare the PCR in the pre-PCR area. Use lab coat and disposable gloves.
- Work on ice or over a cool block. Minimize the time between plate preparation and the start of the assay.
- For each session, it is recommended to include the contamination control (Reaction Blank) and the Positive Control (C+) provided with the kit.

1. Thaw all the kit components before starting the assay. Vortex the Primer Mix vials and carefully mix the Master Mix vials. Centrifuge briefly to collect the volume at the bottom of the tubes.
2. Prepare the following 3 mixes using the volumes indicated in the table below, for n+1 samples:

	R1 mix	R2 mix	R3 mix	Vol. per sample (µL)
Master Mix	MM1	MM2	MM3	10
Primer Mix	PM1	PM2	PM3	8

   Gently mix and centrifuge to ensure that all volume settles to the bottom of the tubes.
3. Pipette 18 µL of these mixes (R1 mix, R2 mix and R3 mix) into the PCR plate/tubes.
4. For each of the 3 reactions, add into each well 2 µL of DNA (recommended concentration between 10 and 200 ng/µL), Positive Control or Reaction Blank. As there are three reactions (R1. R2 and R3), three wells per sample must be used.
5. Seal the plate/tubes using the appropriate sealer and centrifuge briefly to ensure that all the volume settles to the bottom of the well. If possible, make sure there are no bubbles in the wells.
6. Place the plate/tubes in the thermal cycler and set up the thermal cycler amplification programme as described in the following section.

➔ Thermal cycler configuration

1. Set up the following readout channels:
   - FAM channel for FAM-labelled probe detection.
   - HEX/VIC channel for HEX-labelled probe detection.
2. Set up the appropriate amplification profile*, and start the run (*):

	Cycles	Temperature (°C)	Time (mm:ss)	Analysis
Denaturation	1	95	05:00	X
Cycles	40	95	00:10	X
		64	**00:30 (*)**	Single
Cooling	1	15	∞	X

(*) For both Rotor-Gene® Q (Qiagen) and DT-Lite (DNA-Technology) Real-Time PCR thermal cyclers, please set up the following amplification program:

	Cycles	Temperature (°C)	Time (mm:ss)	Analysis
Denaturation	1	95	05:00	X
Cycles	40	95	00:10	X
		64	**00:25 (*)**	Single
Cooling	1	15	∞	X

➔ Disposal

Waste products shall be managed according to local regulations.

10- Results

➔ Results visualization

The analysis of the results is performed with the specific software of the Real-Time PCR instrument being used, and according to the manufacturer's instructions for use.

Once the run is finished, select the linear scale to visualize the amplification curves. It is recommended to check the correct behaviour of the obtained amplification curves:

- An amplification signal is considered positive if a quick and regular increase of fluorescence values (exponential) is observed (sigmoidal amplification) with Ct<35.
- A weak fluorescent, background lineal or exponential signal with Ct>35 should not be considered a positive amplification. This assay allows the detection of some of the alleles included in the group of highly polymorphic HLA alleles, among which little differences in their sequences have been described. Therefore, weak non-specific signals from other similar in sequence but not-detected HLA alleles can be observed in the FAM channel. The appearance of these signals does not invalidate the assay.

A sample is considered positive if it produces an exponential amplification with Ct<35. A sample is considered negative if it produces a non-exponential amplification with low intensity or an exponential amplification with a Ct value >35.

CAUTION!

To determine the Ct value in each channel, adjust the threshold line as follows:

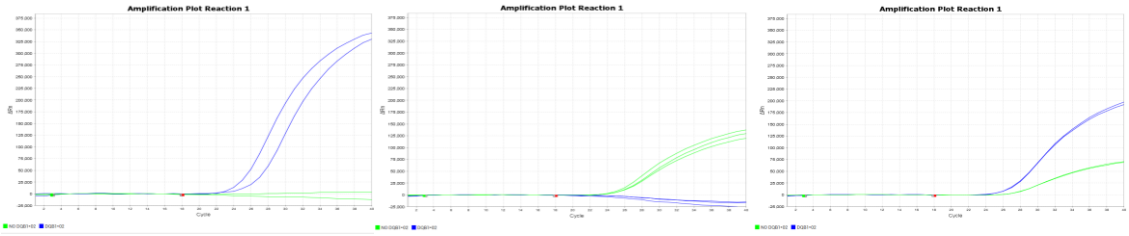
Select linear scale view and choose the region where the fluorescence signal is stable before exponential amplification. Place the threshold line above this background signal, so that it crosses close to the inflection point of the amplification curve. This line should slightly exceed the value of the highest fluorescence obtained with negative samples for the allele detected in this channel.

➔ Results interpretation

The presence of the alleles associated with celiac disease will be determined by the positivity or negativity of the three different reactions.

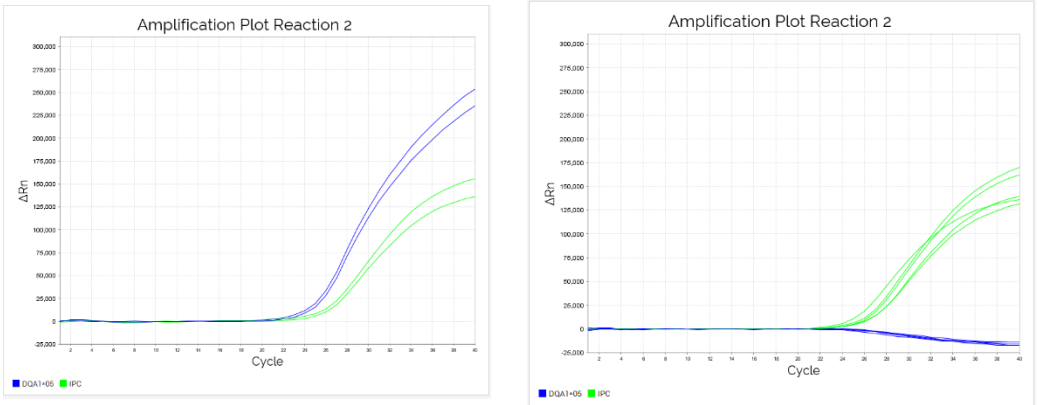
Results obtained with this kit must be interpreted by visualization of the amplification curves in the FAM and HEX channels. Select "linear scale" and determine the absence/presence of sigmoid amplification in each channel.

In Reaction **1**, samples with only an amplification curve in the FAM channel are to be considered as HLA-DQB1*02 homozygous. When only one amplification curve in the HEX channel appears, the sample is to be considered HLA-DQB1*02 negative. If an amplification curve is detected in both FAM and HEX channels, the sample is to be considered as HLA-DQB1*02 heterozygous (one HLA-DQB1*02 allele and any other HLA-DQB1 allele different from DQB1*02).



*Reaction **1**: Amplification curves from HLA-DQB1*02 homozygous (left), DQB1*02 negative (middle) and DQB1*02 heterozygous samples (right). FAM channel (blue), HEX channel (green)*

In Reaction **2**, the β-globin (*HBB*) gene is used as an internal control (IPC). Therefore, all positive and negative samples must generate an amplification curve in the HEX channel to be considered valid, following the criteria established in section "11- Quality Control". Those samples with an amplification curve in both FAM and HEX channels are to be considered HLA-DQA1*05 positive (at least one allele is HLA-DQA1*05 in the analysed sample). Samples with only an amplification curve in the HEX channel are considered HLA-DQA1*05 negative.



*Reaction **2**: Amplification curves from HLA-DQA1*05 positive (left) and DQA1*05 negative (right). FAM channel (blue), HEX channel (green)*

In Reaction **3**, the β-globin (*HBB*) gene is used as an internal control (IPC). Therefore, all positive and negative samples must generate an amplification curve in the HEX channel to be considered valid, following the criteria established in section "11- Quality Control". Those samples with an amplification curve in both FAM and HEX channels are to be considered HLA-DQB1*03:02 positive (at least one allele is HLA-DQB1*03:02 in the analysed sample). Samples that generate amplification curves only in HEX channel are considered HLA-DQB1*03:02 negative.

Expected results for the different possible genotypes are indicated in the next table:

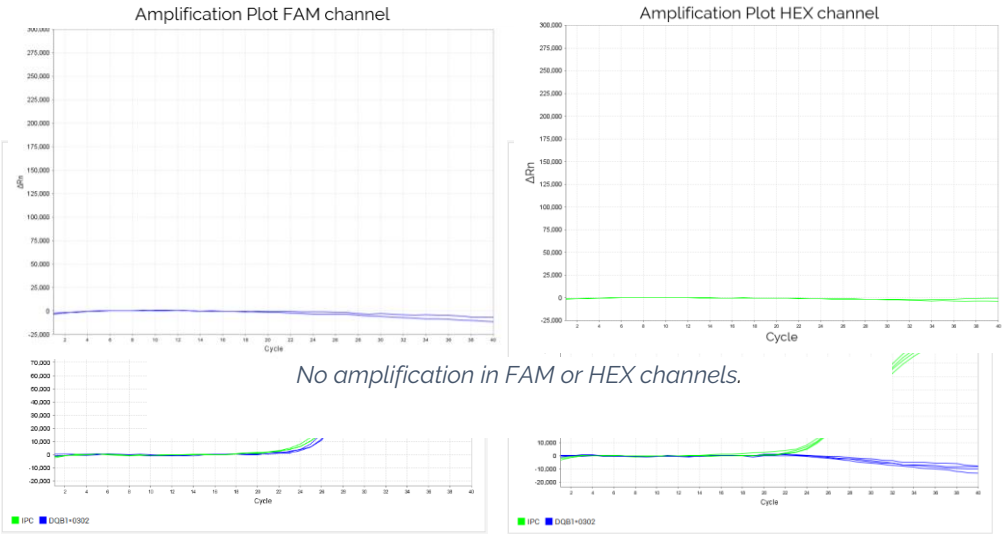
Genotype	Reaction 1		Reaction 2		Reaction 3	
DQ2.5 heterozygous + DQ8	Heterozygous		Positive		Positive	
	FAM+	HEX+	FAM+	HEX+	FAM+	HEX+
DQ2.5 homozygous	Homozygous DQB1*02		Positive		Negative	
	FAM+	HEX-	FAM+	HEX+	FAM-	HEX+
DQ8 positive	Homozygous No DQB1*02		Negative		Positive	
	FAM-	HEX+	FAM-	HEX+	FAM+	HEX+
DQ2.x heterozygous	Heterozygous		Negative		Negative	
	FAM+	HEX+	FAM-	HEX+	FAM-	HEX+
DQ2/DQ8 negative	Homozygous No DQB1*02		Negative		Negative	
	FAM-	HEX+	FAM-	HEX+	FAM-	HEX+

11- Quality control

The kit includes a Reaction Blank and a Positive Control (C+). These controls must be included in each Reaction in each assay. An adequate behaviour of these control samples is a guarantee of the proper performance of the kit.

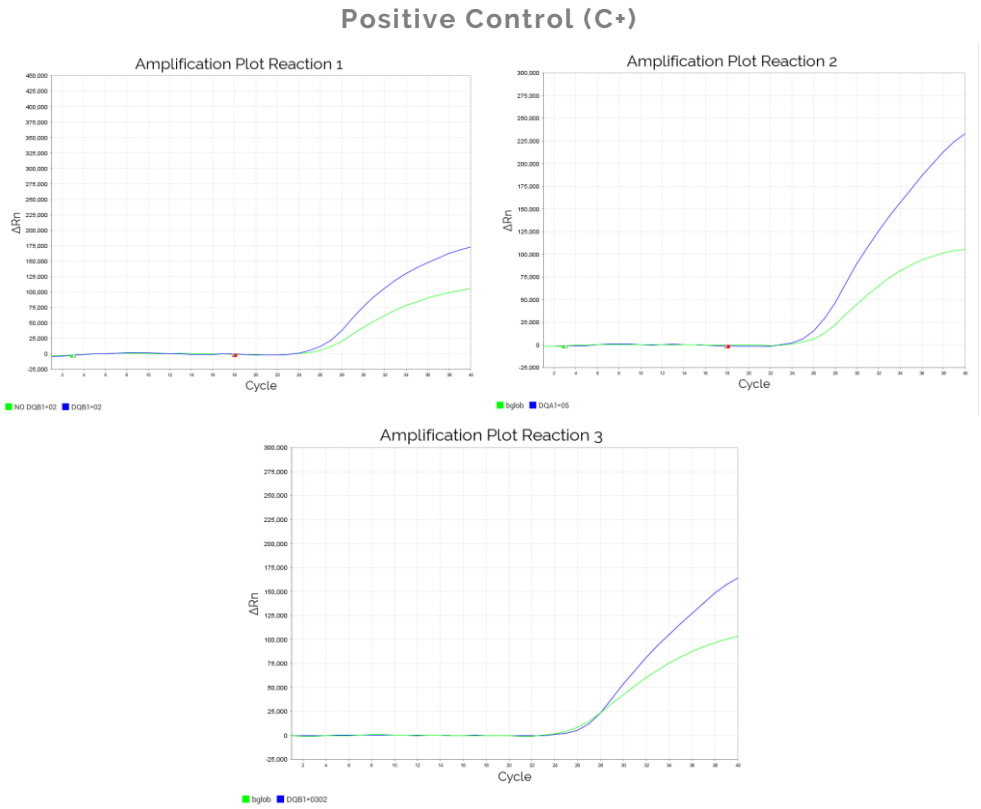
The result of each Reaction is to be considered valid if the following amplification pattern is obtained:

Reaction Blank (RB)



No amplification in FAM or HEX channels.

*Reaction **3**: Amplification curves from HLA-DQB1*03:02 positive (left) and DQB1*03:02 negative (right). FAM channel (blue), HEX channel (green)*



Sigmoidal amplification with a Ct<35 value in both FAM (blue) and HEX (green) channels

The IPC is detected in the HEX channel and used as internal control in Reactions 2 and 3. Therefore, the result of a sample is considered valid if there is, at least, sigmoidal amplification with Ct<35 in the HEX channel in these Reactions.

The result of each Reaction is to be considered invalid and should be repeated if:

- An amplification curve with Ct<35 is observed in FAM and/or HEX channels in the Reaction Blank.
- Non-exponential amplification signal is observed or an amplification signal with Ct>35 appears in the Positive Control.
- There is no amplification curve (Ct<35) in the HEX channel in Reaction 2 and/or 3.
- DNA samples with amplification curves with Ct>35 in FAM and/or HEX channels must be considered doubtful and should be retested performing a new DNA extraction.

If adequate behaviour is observed in the above-mentioned controls, proceed with the interpretation of the samples as indicated in the previous section.

12- Specific operation data

➔ Analytical specificity

Cross-reactivity has been assessed in three independent Genvinset® HLA Celiac kit validation studies, as stated in the "Diagnostic sensitivity and specificity" section.

Due to the highly polymorphic nature of the HLA system, the *in silico* alignment of primers and probes in the most common HLA database (IMGT-HLA) gave a list of detected, no detected and no tested with possible low-intensity signal alleles that can be found at *www. www.bdrdiagnostics.com.* It would be possible that intronic regions of some non-frequent

alleles have not yet been sequenced, so the *in silico* alignment of the kit primers and probes in those areas is unknown. No cross-reaction phenomena with other DNA regions have been reported.

Interference has been studied by means of a bibliographic search. Heparin can inhibit Taq polymerase activity and compete with target nucleic acid, so the collected blood must be treated with other anticoagulants, as stated in the "Sample collection and preparation" section. Some substances found in blood are known as PCR inhibitors: haemoglobin, hemin, bilirubin, bile salts, lactoferrin, immunoglobulin G. The polymerase included in the Genvinset® HLA Celiac kit has demonstrated high resistance to inhibition, and Master Mix composition is designed to deal with interference substances. Nevertheless, the presence of potentially inhibitory substances must be eliminated during DNA extraction and purification protocols. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

➔ Analytical sensitivity

LoD: A dilution assay was performed using different DNA samples (positive and negative for the alleles tested in this assay). Input DNA levels tested ranged from 40 ng to 0.016 ng (**) in a three-fold dilution series. Each level was tested in triplicate. The following data were obtained:

	Reaction 1 DQB1*02	Reaction 2 DQA1*05	Reaction 3 DQB1*03:02
Detection Limit (ng)	0.16	0.49	0.49

- Detection Limit = 0.49 ng (Ct<35)

Upper limit: Samples with different genotypes (positive and negative for the analysed alleles) were assayed in a two-fold dilution series ranging from 400 ng to 6.25 ng (**). Assay performance remained acceptable at all input levels: suitable sigmoidal amplification curves and genotyping calls were accurately made at all levels (with Ct values<35).

(**). DNA concentration was measured using Nanodrop 1000 Spectrophotometer (Thermo Scientific).

➔ Diagnostic sensitivity and specificity

149, 168 and 156 DNA samples were analysed in different laboratories for Reactions 1, 2 and 3, respectively. These samples were previously typed for HLA-DQB1 and HLA-DQA1 loci by a different methodology. The following results were obtained:

		Genvinset® HLA Celiac Reaction 1		
	DQB1*02	Homozygous	Heterozygous	Negative
Previous information	Homozygous	33	0	0
	Heterozygous	0	52	0
	Negative	0	0	64

		Genvinset® HLA Celiac Reactions 2 and 3			
		Reaction 2 DQA1*05		Reaction 3 DQB1*03:02	
	Genotype	Pos.	Neg.	Pos.	Neg.
Previous information	Positive	71	0	58	0
	Negative	0	97	0	98

There is a 100% match in the results obtained with Genvinset® HLA Celiac and the genotypes previously obtained with a different methodology.

➔ Accuracy

Repeatability studies consist in the measurement of within-run variability, through the analysis of replicas of every kind of sample that can be determined with the kit. Each sample was analysed in duplicate.

Genvinset® HLA Celiac showed 100% repeatability.

A study to determine the reproducibility of the reagent was performed. It allows to estimate the variability between runs, lots and operators. Three different lots were analysed in three different instruments:

Genvinset® HLA Celiac showed 100% reproducibility.

➔ Trueness

The trueness of the analytical procedure of Genvinset® HLA Celiac is assessed by comparison with a reference method. The study was developed as an internal validation of the reagent, in which trueness has been demonstrated with a 100% value. See the "Diagnostic Sensitivity and Specificity" section.

13- Procedure limitations

- The method detects the HLA-DQB1*02/03:02 and DQA1*05 included in the "HLA alleles detected_GVS-DQ" document uploaded at *www.bdrdiagnostics.com*. Due to the highly polymorphic nature of the HLA alleles, weak signals from other alleles similar in sequence but not detected could appear in the FAM channel.
- Mutations or polymorphisms at annealing primer/probe sites are possible and may result in the lack of allele definition. Other technologies could be necessary to resolve the typing.
- All the recommendations mentioned in this document should be carefully followed. Any performances that do not meet such indications, can lead to poor results.
- Do not use the kit if there are any suspicions of possible loss of reactivity, contamination, external box deterioration or any other incidence that might affect the kit's performance.
- All Genvinset® reagent manipulations must be done according to good laboratory practice, being adapted to local regulations.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiration date. Discard expired reagents according to applicable regulations.
- The Real-Time PCR thermal cycler must be calibrated according to the manufacturer´s recommendations and should be used accordingly to the manufacturer's instructions.
- Due to the complexity of HLA typing, data and result interpretation must be revised by qualified personnel.
- This serves as an auxiliary tool for the diagnosis of patients with suspected celiac disease. Use these results in conjunction with clinical data and results of other tests performed on the patient.

14- Troubleshooting guide

➔ No amplification signal is detected in any sample (neither in positive controls) or the intensity is very low

- The Real-Time PCR instrument is not correctly programmed. The thermal profile is not correct/the reading channels are not correctly configured/the selected fluorophores are not suitable.
    - Check that the instrument has been programmed correctly.
- The positions of the samples and controls indicated during the preparation of the assay do not match the positions in which they have been placed in the device.
    - Correctly assign the position of the samples.
- The reagent does not work properly.
    - Ensure that the kit is stored at an appropriate temperature (between -30°C and -18°C) and protected from light. Avoid unnecessary freeze/thaw cycles. Do not use it beyond the expiry date.
- The indicated amounts of each of the reagents have not been added to the reaction mix.
    - Check the volume of each component added to the mix.
- The consumables used are not compatible with the equipment being used.
    - Make sure that the right consumables have been used (compatible with the PCR instrument used).

➔ No signal detected in clinical samples (signal appears in positive control)

- Poor quality of the DNA used.
    - Check the Absorbance ratio 260/280 and discard poor-quality samples. Avoid the presence of inhibitors (heparin, haemoglobin, hemin, bilirubin, bile salts, lactoferrin, immunoglobulin G). Repeat sampling and DNA extraction.
- Inadequate DNA concentration.
    - Adjust the DNA concentration to the recommended concentration range.
    - Check that the DNA concentration is above the limit of detection.
- Inhibition of amplification.
    - Collect whole blood in EDTA or citrate tubes.
- No sample was added.
    - Repeat the assay making sure that samples have been added.

➔ Signal detected in the negative control

- Pipetting error
    - Change the pipette tip each time DNA is added to a well. Check that the sample added to the well corresponds to what is written on the worksheet.
- Contamination of the Primer Mix/Master Mix/Reaction Blank vial.
    - Repeat the assay with fresh aliquots.
- The PCR preparation area is contaminated.
    - Clean surfaces, instruments, lab coats, and change consumables and reagents. Repeat the assay.

➔ Fluorescence intensity varies between samples or abnormal amplification curves

- Dirtiness outside the reaction tube interferes with the detection of fluorescence.

- Clean the equipment thoroughly. Check that the outside of the tubes/plate is clean. Handle the plate/tube with gloves.

- The volume is not at the bottom of the well or there are bubbles.
  - Centrifuge the tubes/plate before placing them in the thermal cycler.
  - Check if there are any bubbles. If so, perform a brief spin to remove them.
- The plate/tubes have not been closed properly.
  - Repeat the assay checking that the tubes/plates have been sealed correctly.
- DNAs with different concentrations have been used or the sample used contains a reaction inhibitor.
  - Repeat the sampling and DNA extraction.
- Presence of polymorphisms or mutations at the probe/primer binding sites.
  - Contact our Technical Support department at *customersupport @bdrdiagnostics.com*

15- References

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16- Notice to purchaser

- This product has been developed for *in vitro* diagnostic purposes.
- When the kit is used within the territory of any Member State of the European Union, should there be any serious incident regarding the use of the kit, the user must report it to the manufacturer (*regulatory@bdrdiagnostics.com)* and the Competent Authority of their country and/or the patient´s country. Serious incident reporting for all other countries must be conducted according to local requirements for each country.

- The Summary of Safety and Performance (SSP) is uploaded into EUDAMED database, and it is accessible to the users of the kit (*https://ec.europa.eu/tools/eudamed/#/screen/home*)
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17- Changes control

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
Rev. 12	Change in the reaction volume. Change in the thermal cycler amplification protocol. Change in compositions of Primer and Master Mix.
Rev. 13	Correction of typos and translation mistakes. Phone number and e-mail BDR addition. Change in Positive Control volume. Addition of UDI-DI codes. Change in the validated Real-Time PCR cyclers. Notice that The Summary of Safety and Performance (SSP) is uploaded into EUDAMED database, and it is accessible to the users of the kit.

18- Explanation of symbols used on the labels

IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalogue number	Σ	Contents sufficient for <n> tests
LOT	Lot number		Manufacturer
	Temperature limit		Keep away from sunlight
CONTROL +	Positive control		Consult electronic Instructions For Use document
CE	This product fulfills the requirements of Directive 98/79/EC on *in vitro* diagnostic medical device		