

GenVInSet

HLA-B27v4

# Instructions for use

Kit for allele detection in the  
HLA-B27 group

For In Vitro Diagnostic Use

Referencia GVS-B2704-48 (48 tests)  
GVS-B2704-24 (24 tests)

Store from  $-18$  to  $-30^{\circ}\text{C}$



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# Index

Intended use	3
Summary and explanation	4
Procedure principles	5
Kit contents	6
Kit storage	7
Materials required but not supplied	8
Sample collection and preparation	9
Procedures	10
A) PCR preparation	
B) Thermal cycler configuration	
Results	13
Quality control	14
Specific operation data	15
HLA-B*27 (IMGT-HLA 3.37.0) family alleles detected by Genvinset®HLA-B27v4 kit	16
Procedure limitations	19
Troubleshooting guide	20
References	22
Notice to purchaser	23
Changes to version 03	24
Explanation of symbols used on the labels	25

## Intended use

GENVINSET® HLA-B27v4 is a kit for HLA-B\*27 group allele determination by Real time PCR using Taqman® probes technology.

## Summary and explanation

The major histocompatibility complex (MHC) is the genetic region that contains the most polymorphic loci of the genome involved in the mechanism of antigen presentation and, as such, defines the general immunological response.

Within the MHC, the allelic family HLA-B\*27 is part of the HLA-B locus and is present in the 3-8% of the caucasian population. Despite this frequency the interest in this allelic family relies in its relation with different rheumatic diseases called spondyloarthropathies, among which it is worth highlighting the ankylosing spondylitis (AS).

Around 90% of the patients with AS are HLA-B\*27 positive. Other related autoimmune diseases are juvenile rheumatoid arthritis (80% of patients), and the Reiter syndrome or reactive arthritis (50-80%).

The HLA-B\*27 is also present in 50% of patients who suffer from spondylitis with inflammatory bowel disease or plaque psoriasis. The HLA-B\*27 allele is not the root cause of these pathologies, but it shows a higher prevalence in affected patients.

4

In HLA-B\*27 positive patients, the antigen (structural protein) is present in the membrane of all nucleated cells, including leukocytes.

If HLA-B\*27 positive patients show symptoms like chronic pain, inflammation and/or degenerative bone changes (visible through radiology tests), it is very likely that they suffer from AS, Reiter syndrome or another autoimmune pathology associated with this antigen. Chances are higher in young male patients who start having symptoms before the age of 40.

The presence of the HLA-B\*27 antigen can also be seen in other autoimmune pathologies like isolated acute anterior uveitis, idiopathic spondyloarthropathies and enteropathic synovitis.

The absence of the HLA-B\*27 antigen does not rule out the autoimmune pathology, as 10% of the AS carriers and 40-50% of Reiter syndrome patients are HLA-B\*27 negative.

Moreover, if a patient does not show any of the associated symptoms, the detection of the HLA-B\*27 antigen per se does not allow to determine the presence of an autoimmune disease, its progression or prognosis.

## Procedure principles

The detection method used by Genvinset® is based on the Real Time PCR technology, monitored with Taqman® probes.

The Genvinset® HLA-B\*27 kit allows for the detection of all the alleles belonging to the HLA-B\*27 family(\*) and a control gene ( $\beta$ -Globin), used to verify the results of the assay.

Each pair of primers is complementary to two DNA sequences, located in cis, thus making of the current kit a high resolution technique with high sensitivity, specificity and reproducibility.

(\*) See section 'Procedure limitations' (page 21).

## Kit contents

### GVS-B2704-48 (48 tests)

- GVS-B27-PMv4: 2 vials x 110 uL Primer Mix (PM)
- GVS-B27-MM: 2 x 138 uL Master Mix (MM)
- GVS-B27-C+: 1 x 5uL Positive Control (C+)
- GVS-RB: 1 x 100 uL Reaction Blank (RB)

### GVS-B2704-24 (24 tests)

- GVS-B27-PMv4: 1 vial x 110 uL Primer Mix (PM)
- GVS-B27-MM: 1 vial x 138 uL Master Mix (MM)
- GVS-B27-C+: 1 vial x 5 uL Positive Control (C+)
- GVS-RB: 1 vial x 100 uL de Reaction Blank (RB)

## Kit storage

In order to ensure a proper performance, reagents should be stored from -18°C to -30°C until their expiration date, indicated on the label of the vial. Do not perform more than 3 freeze/thaw cycles to the Primer Mix (GVSB27PMv4) and Master Mix (GVS-B27-MM) vials, as this could decrease the sensitivity of the assay and impair results.

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

# Materials required but not supplied

## General

- Gloves
- Lab coat

## Consumables

- Filter tips (P200, P200 and P10)
- 1.5 mL Autoclaved Tubes
- q-PCR instrument specific reagents (in the case of using RotorGene Q, only 0.1 ml tubes are allowed).

## Equipment

- q-PCR instrument, with FAM and HEX/VIC detection channels. The following devices 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 & P10)



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

### **i** Caution

All biological and blood samples should be treated as possibly infectious. All the corresponding basic (universal) precautions should be taken. Samples should always be handled wearing the appropriate personal protection equipment.

# Procedure

## A) PCR preparation

### **i** Precautions

- Thaw all components of the kit before starting the assay, mix and centrifuge them.
- Work on ice or over a cool block.
- The PCR should be setup in the Pre-PCR area.
- Use only filtered tips and autoclaved tubes 1.5 mL.
- Use gloves and lab coat at all times.
- In each session it is recommended to test the contamination control (Reaction Blank) and the Positive Control (B27+) included in the kit, as well as a B\*27 negative sample.

10

1. Thaw the samples. Vortex (or gently mix with the fingers).
2. Prepare the following mix for n+1 samples:

	Vol. per sample (µL)
Master Mix	5
Primer Mix	4

3. Pipette 9 µl of the mix in each well. Then add 1 µl of DNA, Positive Control or Reaction Blank in the corresponding well.

4. Seal the plate or the tubes. Perform a brief spin to ensure that all the volume settles to the bottom of the tube.
5. Place the plate in the thermal cycler and start the following program.

#### B) Thermal cycler configuration

1. Set up the following amplification program:

	Cycle Number	Temperature (°C)	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 as indicated below:

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. Select the tab “Advanced” in the window New Run, and click “New”.
- b. Select the type of rotor used (only 0.1 mL tubes accepted, see section `Required materials not supplied`, page 8). Select the “Locking Ring Attached” box and continue by clicking “Next”.
- c. Set the “Reaction Volume” as 10 µL, and identify the operator and the samples.
- d. Click on “Edit Profile” and set up the amplification program (see section 8.C.1). Select the step 60 sec at 62 °C, and click on “Acquiring to Cycling A”. Set “Green” and “Yellow” as the fluorescence acquisition channels. Press “OK”. Click on “OK” to accept and close the “Edit Profile” window.
- e. Click “Gain Optimisation” in the “Run New Wizard” dialog box to open the “Auto-Gain Optimization Setup” window. Scroll down the “Channel Settings” menu and select “Acquiring Channels”. Then click on “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
- f. Check the box “Perform Optimisation Before 1st Acquisition”, and click on “Close”.
- g. Select “Next” and then “Start Run” in the “New Run Wizard” window.

## Results

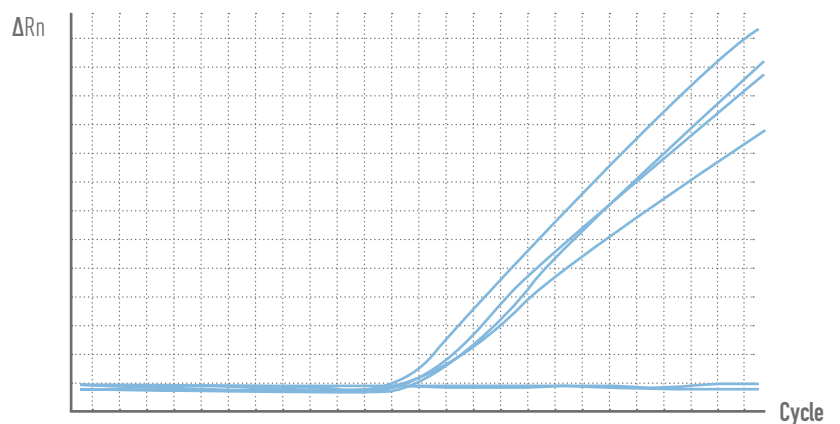
Genvinset® HLA-B\*27v4 constitutes a qualitative technique to determine the presence or absence of the HLA-B\*27 family of alleles.

It is not necessary to select any passive reference.

The interpretation of results should be done as follows:

### B\*27 results

Selecting the FAM channel in the Amplification Plot, a graphic similar to the following one can be displayed:

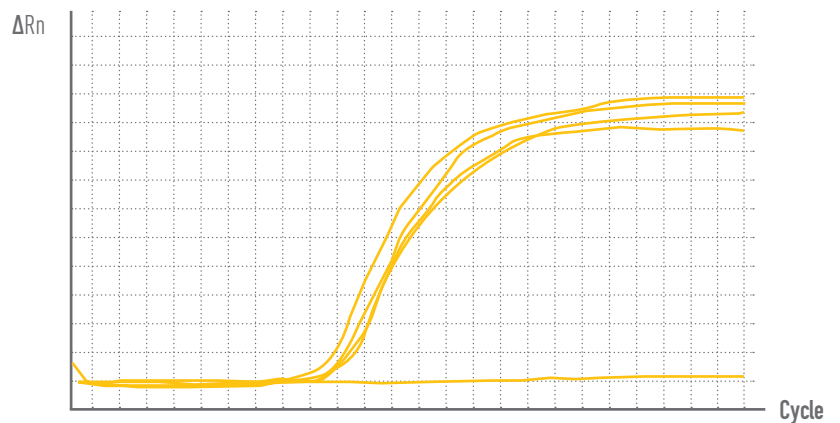


13

Samples generating an amplification curve are to be considered as B\*27 positive and can be identified by a numeric value called Crossing Point (Cp), which corresponds to the cycle in which fluorescence can be detected.

### $\beta$ -globin results

Selecting the VIC/HEX channel in the Amplification Plot, a graphic similar to the following one can be displayed:



Samples generating an amplification curve are positive for the internal control ( $\beta$ -globin).

## Quality control

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

It is recommended to perform a contamination control by replacing the DNA for the negative control supplied with the kit and a positive control (HLA-B\*27 positive sample).

The following criteria have to be taken into account in order to validate an assay:

- The Reaction Blank should report negative results in both FAM and VIC/ HEX channels. An amplification curve with a  $C_p > 35$  value should be considered as negative. A  $C_p < 35$  value is associated with a contamination in the Reaction Blank and, therefore, all results from the experiment should be discarded.
- A positive control sample must render positive results for both B\*27 and for  $\beta$ -globin.
- DNA samples should always be positive for  $\beta$ -globin ( $C_p < 35$ ).
- DNA samples generating results with  $C_p > 35$  for  $\beta$ -globin and/or B+27 genes must be considered as doubtful and should be retested performing a new DNA extraction.

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

# Specific operation data

## 1. Analytical specificity

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

## 2. Analytical sensitivity

A serial dilution assay was performed using two DNA samples (one B\*27 positive and one B\*27 negative), at a concentration of 38,1 and 109,9 ng/μL, respectively. Both were obtained with a conventional DNA extraction system. The following results for the analytical sensitivity of the test were obtained:

- DNA sample obtained by conventional extraction system:  
Detection Limit = 0.6 ng/μL (\*)

(\*) Cp < 35

## 3. Diagnostic sensitivity and specificity

All the 258 tested samples were validated (positive amplification of the β-Globin control gene). Among those, 44 were found to be B\*27 positive.

15

GENVINSET® HLA-B27v4			
	Samples	B27+	B27-
SSO/ SBT	B27 +	44	0
	B27 -	0	214

There is a 100% match in the results obtained with Genvinset(R) HLA-B27v4 and the previously obtained genotype with other commercial kits.

# HLA-B\*27 (IMGT-HLA 3.37.0) family alleles detected by Genvinset® HLA-B27v4 kit

<b>B*27:01</b>	B*27:05:02:11	B*27:05:29	B*27:09
<b>B*27:02:01:01</b>	B*27:05:03	B*27:05:30	B*27:10
B*27:02:01:02	B*27:05:04	B*27:05:31	B*27:11
B*27:02:01:03	B*27:05:05	B*27:05:32	B*27:12:01:01
B*27:02:01:04	B*27:05:06	B*27:05:33	B*27:12:01:02
B*27:02:01:05	B*27:05:07	B*27:05:34	B*27:13
B*27:02:02	B*27:05:08	B*27:05:35	B*27:14
B*27:02:03	B*27:05:09	B*27:05:36	B*27:15
B*27:02:04	B*27:05:10	B*27:05:37	B*27:16
B*27:02:05	B*27:05:11	B*27:05:38	B*27:17
<b>B*27:03</b>	B*27:05:12	<u>B*27:05:39</u>	B*27:18
<b>B*27:04:01</b>	B*27:05:13	B*27:05:40	B*27:19
B*27:04:02	B*27:05:14	B*27:05:41	B*27:20
B*27:04:03	B*27:05:15	B*27:05:42	B*27:21
B*27:04:04	B*27:05:16	B*27:05:43	<u>B*27:23</u>
B*27:04:05	B*27:05:17	B*27:05:44	B*27:24
B*27:04:06	B*27:05:18	B*27:05:45	B*27:25
<b>B*27:05:02:01</b>	B*27:05:19	B*27:05:46	B*27:26
B*27:05:02:02	B*27:05:20	<b>B*27:06:01:01</b>	B*27:27
B*27:05:02:03	<u>B*27:05:21</u>	B*27:06:01:02	B*27:28
B*27:05:02:04Q	B*27:05:22	<b>B*27:07:01</b>	B*27:29
B*27:05:02:05	B*27:05:23	B*27:07:02	B*27:30
B*27:05:02:06	B*27:05:24	B*27:07:03	B*27:31
B*27:05:02:07	<u>B*27:05:25</u>	B*27:07:04	B*27:32
B*27:05:02:08	B*27:05:26	B*27:07:05	B*27:33
B*27:05:02:09	B*27:05:27	B*27:07:06	B*27:34
B*27:05:02:10	B*27:05:28	<b>B*27:08</b>	B*27:35



# HLA-B\*27 (IMGT-HLA 3.37.0) family alleles detected by Genvinset® HLA-B27v4 kit

B*27:36	B*27:64N	B*27:90:04	B*27:118
B*27:37	B*27:65N	<u>B*27:91</u>	B*27:119
B*27:38	B*27:66N	B*27:92	B*27:120
B*27:39	<u>B*27:67</u>	B*27:93	B*27:121
B*27:40	B*27:68	B*27:94N	B*27:122
B*27:41	B*27:69	B*27:95	B*27:123
B*27:42	B*27:70	B*27:96:01	B*27:124
B*27:43	B*27:71	B*27:96:02	B*27:125
B*27:44	B*27:72	B*27:97	B*27:126
B*27:45	B*27:73	B*27:98	B*27:127
B*27:46	B*27:74	B*27:99	B*27:128
B*27:47	<u>B*27:75</u>	B*27:100	B*27:129
B*27:48	B*27:76	B*27:101	B*27:130
B*27:49	B*27:77	B*27:102	B*27:131
B*27:50:01	B*27:78	B*27:103	B*27:132
B*27:50:02	B*27:79	B*27:104	B*27:133
B*27:51	B*27:80	B*27:105	B*27:134
<u>B*27:52</u>	B*27:81	B*27:106	B*27:135
B*27:53	B*27:82	B*27:107	B*27:136
B*27:54	B*27:83	B*27:108	B*27:137
B*27:55	B*27:84	<u>B*27:109</u>	B*27:138
B*27:56	<u>B*27:85</u>	B*27:110	B*27:139
B*27:57	B*27:86	B*27:111	<u>B*27:140</u>
B*27:58	B*27:87	B*27:112	B*27:141
B*27:59N	B*27:88	B*27:113	B*27:142
B*27:60	B*27:89	B*27:114	B*27:143
B*27:61	B*27:90:01	B*27:115	B*27:144
B*27:62	B*27:90:02	B*27:116	B*27:145
B*27:63	B*27:90:03	B*27:117	B*27:146

# HLA-B\*27 (IMGT-HLA 3.37.0) family alleles detected by Genvinset® HLA-B27v4 kit

B*27:147	B*27:176N	B*27:205
B*27:148	B*27:177	B*27:206
B*27:149	<u>B*27:178</u>	B*27:207
B*27:150	B*27:179	B*27:208
B*27:151	B*27:180	B*27:209
B*27:152	B*27:181	B*27:210
B*27:153	B*27:182	B*27:211
B*27:154	B*27:183	B*27:212N
B*27:155	B*27:184	B*27:213
B*27:156	B*27:185	B*27:214
<u>B*27:157</u>	B*27:186	B*27:215N
B*27:158	B*27:187	
B*27:159	B*27:188	
B*27:160	<u>B*27:189</u>	
B*27:161	B*27:190	
B*27:162	B*27:191	
B*27:163	B*27:192	
B*27:164	B*27:193	
B*27:165	B*27:194	
B*27:166	B*27:195	
B*27:167	B*27:196	
B*27:168	B*27:197	
B*27:169	<u>B*27:198</u>	
B*27:170	B*27:199	
B*27:171	B*27:200	
B*27:172	B*27:201	
B*27:173	B*27:202	
B*27:174	B*27:203	
B*27:175	B*27:204	

## Procedure limitations

- The current method allows for the detection of all HLA-B\*27 group of alleles, except those specified in the previous section (IMGT-HLA 3.37.0).
- The presence of mutations or polymorphisms at the primer/probes annealing sites is possible and may result in the lack of allele definition. Alternative technologies could be necessary to resolve the typing.
- All the aforementioned conditions for the setup of the PCR should be carefully controlled. Any performances that do not meet such indications, can lead to poor results.
- All Genvinset® components manipulation must be done according to general lab best practices and be adjusted to local regulations, like the EFI standard (European Federation of Immunogenetics).
- The q-PCR thermal cycler must be calibrated according to the manufacturers' recommendations and should be used in accordingly to the manufacturer's instructions.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiration date.
- 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 kits performance.
- Due to the complexity of HLA typing, data and result interpretation should be revised by qualified personnel.
- Discard expired reagents according to applicable regulations.

# Troubleshooting guide

## Problem

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

## Reaction Blank (H<sub>2</sub>O) is positive

- **Primer Mix/Master Mix/Reaction Blank contamination**
  - Repeat the experiment with new Primer Mix/Master Mix/Reaction Blank aliquots.
  - Handle the kit components always according to accepted lab practices in order to avoid contamination.
  - Verify manipulation and storage conditions.
  - Discard contaminated reagents.
- **Pre-PCR area is contaminated**
  - Confirm that all necessary precautions in the pre-PCR area have been followed.
  - Check for possible contamination problems in other PCR techniques.
  - Confirm suitability of the used reagents (1.5 mL tubes, pipette tips).
- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

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

- **Samples with very low DNA concentration**
  - Check the DNA concentration and repeat the extraction if necessary.
- **DNA samples with high concentration**
  - Perform the assay using diluted samples.

## Fluorescence intensity too low

- **Kit degradation (Primer Mix or Master Mix vials)**
  - Check that the storage of the kit is correct, reviewing both proper temperature conditions and light exposure (which should be avoided).
  - Avoid performing more than 3 thaw/freezing cycles to the reagents.

- Aliquote the reagents if necessary.
- Repeat the test with new reagents.

#### Negative control sample is positive

- **Cross contamination**
  - Always handle the kit components following all necessary practices to avoid contamination.
- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

#### Positive control sample is negative

- **Pipetting error**
  - Check that the sample added corresponds to the one indicated on the worksheet.

#### Fluorescence intensity varies

- **The dirtiness on the outside of the tube walls interferes with the signal.**
  - Handle all consumables wearing gloves.
- **Volume is not settled to the bottom of the well or there are bubbles**
  - Perform a brief centrifugation to ensure that the volume settles to the bottom of the well and to remove all bubbles.
- **Pipetting error**
  - Check that the correct volume has been added.

#### There is no fluorescence signal

- **Incorrect reading channels selected**
  - Set the appropriate reading channels.
- **Pipetting error or reagent absence**
  - Control the pipetting and the reaction setup.
  - Repeat the PCR.
- **No reading channel was selected in the thermal cycler program.**
  - Check and modify the thermal cycler program.

## References

1. "HLA-B27 Genotyping by Fluorescent Resonance Emission Transfer (FRET) Probes in Real-Time PCR". Rosa Faner, Natàlia Casamitjana, Roger Colobran, Anna Ribera, Ricardo Pujol-Borrell, Eduard Palou, and Manel Juan. *Biotechniques*. 1996 Jun;20(6):1012-4, 1016, 1018-20.
2. "Optimization of Dnase I removal of contaminating DNA from RNA for use in quantitative RNA-PCR." Huang Z, Fasco MJ, Kaminsky LS. *School of Public Health, State University of New York, New York 12201-0509, USA*.

## Notice to purchaser

- This product has been developed for in vitro diagnostics purposes.
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## Changes to version 03

Version	Description of the modification
Rev. 03	Reduction of number of PCR cycles



## Explanation of symbols used on the labels



For in vitro diagnostic use



This product fulfills the requirements of Directive 98/79/EC on in vitro diagnostic medical device



Catalogue number



Lot number



Expiration date



Contents sufficient for <n> tests



Manufactured by



Store at



Keep away from sunlight



Positive control