GenVinSet

HLA-B27

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 –30ºC

CE 0086

Rev 01 / 20-07-2018

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GenVinSet

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Intended use

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

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HLA-B27

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 B27 is part of the HLA-B locus and presents a frequency between 3 and 8% of the caucasian population. Despite this frequency the interest in this allelic family relies in its relation with different rheumatic diseases called spondyloarthropathies, from which we can highlight ankylosing spondylitis (AS).

Around 90% of the patients with AS are HLA B27 positive. Other related autoimmune diseases are Juvenile rheumatoid arthritis (80% of patients), and the Reiter syndrome or reactive arthritis (50-80%).

The HLA B27 is also present in 50% of the patients with inflammatory bowel disease with spondylitis and plaque psoriasis with spondylitis. HLA B27 is not the root cause of these pathologies, but there is a higher prevalence of this antigen in affected patients.

A person can be HLA-B27 positive or negative. If positive, the antigen HLA B27 (structural protein) is present in the membrane of all the organisms nucleated cells, including Leukocytes.

If, albeit the HLA B27, there are symptoms like chronic pain, inflammation and/or degenerative bone changes (visible through radiology), it's very likely that the patient has AS, Reiter syndrome or another autoimmune pathology associated with HLA B27. This is more likely if the patient is a young male which started having symptoms before the age of 40.

The presence of the HLA B27 antigen can also be seen in other autoimmune pathologies like isolated acute anterior uveitis, idiopathic spondyloarthropaties and enteropathic synovitis.

The absence of the HLA B27 antigen makes it less likely for the symptoms to be due to an autoimmune pathology associated with this antigen. (There are exceptions, as 10% of the AS carriers and 40-50% of Reiter syndrome carriers are HLA B27 negative)

These days, being able to detect the HLA B27 is not an indication that an autoimmune disease is developing, as if the patient does not present any of the associated symptoms, the presence of the HLA B27 does not allow us to determine which disease it has, how fast it will evolve, its severity, its prognosis or the degree of organic involvement.

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Procedure principles

The detection method used by Genvinset is based in a primer specific PCR, which anneal to all the alleles of the HLA-B27 family (*) monitored with Taqman® probes.

At the same time the method amplifies and detects a control gene (ß-globin) to verify the assay's result.

This technique provides high resolution because each pair of primers recognizes 2 DNA areas situated in cis, resulting in high sensitivity, specificity and reproducibility.

(*) See section 'Limitations of the procedure' (page 21)

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Kit contents

Reference GVS-B2704-48 (48 tests)

- GVSB27PMv4: 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)

Reference GVS-B2704-24 (24 tests)

- GVSB27PMv4: 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)

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Kit storage

All of the kit's reagents should be stored from -18ºC to -30ºC and they are stable at this temperature until its expiration date, as indicated in the bottle. Do not perform more than 3 freezing/thawing cycles to the Primer Mix and Master Mix vials (GVSB27PMv4, GVS-B27-MM) as this could reduce the assays sensitivity and change 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, P200 and P10)
- 1.5 mL Autoclaved Tubes
- RT-PCR instrument specific reagents (when using Rotor Gene-Q only use 0.1mL. tubes).

Equipment

- RT-PCR instrument. The next 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®
- Vortex
- Pipettes (P200, P100 & 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.

This technique is compatible with several conventional extraction systems. Before validating results with any extraction systems, perform a technique validation test (assay).

Caution

All biological and blood samples should be treated as possibly infectious. When manipulating them, observe all basic (universal) precautions. All sample manipulation should be done with gloves and appropriate protection.

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Usage procedures

A) PCR preparation

Precautions

- Unfreeze all of the kits components before starting the assay, mix and centrifuge.
- Work over ice or a cold block.
- The PCR should be assembled 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 negative B27 sample.

1. Take the samples out of the freezer. Vortex (or use finger knocks).
2. Prepare the mix of Primer Mix and Master Mix to n+1 samples:

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

3. Pipette 9 µL of this mix on the wells over the cold holder and add 1 µL of DNA, or Positive Control or Reaction Blank, in the contamination control well.

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4. Seal the plate with convenient sealer, spin down the volume by centrifuging 1 min. 360 x g.

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

B) Thermal cycler configuration

1. Set up the next amplification program:

2. Set up the reading channels

	Cycle Number	Temperature (ºC)	Time (mm:ss)	Ramp (%)	Analysis
Desnaturalization	1	95	05:00	100	X
Cycles	50	95	00:15	100	X
		64	1:00	100	Single
Cooling	1	15	∞	100	X

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. Type the "Reaction Volume" as 10 uL, and identify the operator and the sample.

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

e. Clic "Gain Optimisation" in the "Run New Wizard" dialog box to open the "Auto-Gain Optimization Setup" window. In the scroll menu of "Channel Settings" select "Acquiring Channels" and

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then "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. Activate the option "Perform Optimisation Before 1st Acquisition", and click "Close".

g. Select "Next" and then "Start Run" in the "New Run Wizard" window.

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Results

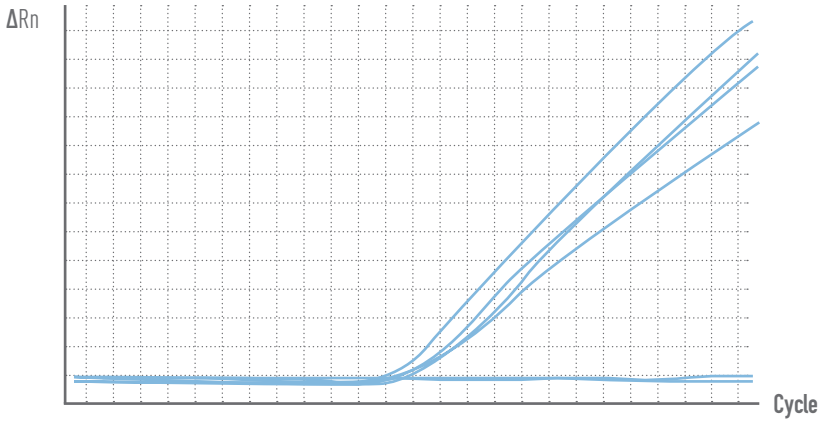
GENVINSET B27 is a qualitative technique to identify presence or absence of the HLA-B27 family alleles.

It is not necessary to select any passive reference.

The results of this technique can be obtained as follows:

B27 results

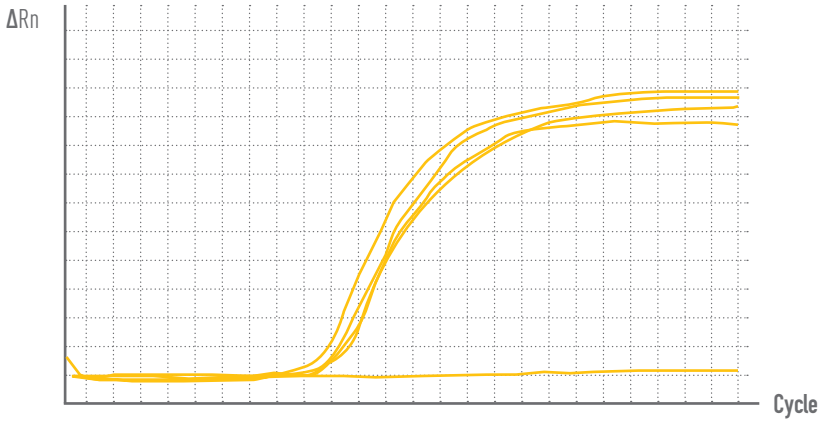
Selecting FAM channel in Amplification Plot, we can see next graphic:



Samples generating an amplification curve are B27 positive and they are identified by a numeric value called Crossing Point (Cp) corresponding with the cycle in that fluorescence can be detected.

ß-globin results

Selecting VIC/HEX channel in Amplification Plot, we can see next graphic:



Samples generating an amplification curve are positive for internal control (ß-globin).

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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 perform a contamination control by replacing the DNA for the negative control supplied in the kit and a positive control (sample with HLA-B27 typing).

The following criteria should be known for the assay to be considered valid:

- The contamination control (Reaction Blank) must provide negative results both for B27 and for ß-globin. Cp (Crossing Point) values >35 would be consider as negative result. Cp values <35 inform us about a contamination in the session so results should be discarded.
- A positive control sample must provide positive results for both B27 and for ß-globin.
- DNA samples should always be positive for ß-globin (Cp<35).
- DNA samples generating results with Cp>35 for ß-globin and/or B27 loci must be considered as doubtful and must be retested performing a new extraction of DNA.

The assay must be made 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.

2. Analytical sensitivity

Once performed a dilution assay using 1:4 serialized dilutions of two DNA samples, one of them B*27+ and the other on B*27-, obtained by a conventional extraction system, at a concentration of 24.0 and 31.1 ng/uL respectively, the following results were obtained as for analytical sensitivity of the B27 allele detection:

- DNA sample obtained by conventional extraction system:
  Detection Limit = 0.4 ng/µL (*)

  (*) Cp < 35

3. Diagnostic sensitivity and specificity

Of the 75 samples tested, all of them were validated (positive amplification of the ß -globin control gene), and 19 of them were found to be B27 positive.

	GENVINSET B27v4		
	Samples	B27+	B27-
SSO/ SBT	B27 +	19	0
	B27 -	0	56

There is a 100% match in the results obtained with GENVINSET B27v3 and the typing previously obtained with the SSO (Sequence Specific Oligonucleotide probes) or SBT (Sequence Based Typing) methodologies.

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HLA-B27 (IMGT-HLA 3.31.0) family alleles detected by GENVINSET B27

B*27:01	B*27:05:09	B*27:07:03	B*27:31
B*27:02:01:01	B*27:05:10	B*27:07:04	B*27:32
B*27:02:01:02	B*27:05:11	B*27:07:05	B*27:33
B*27:02:01:03	B*27:05:12	B*27:07:06	B*27:34
B*27:02:01:04	B*27:05:13	B*27:08	B*27:35
B*27:02:01:05	B*27:05:14	B*27:09	B*27:36
B*27:02:02	B*27:05:15	B*27:10	B*27:37
B*27:02:03	B*27:05:16	B*27:11	B*27:38
B*27:02:04	B*27:05:17	B*27:12:01:01	B*27:39
B*27:03	B*27:05:18	B*27:12:01:02	B*27:40
B*27:04:01	B*27:05:19	B*27:13	B*27:41
B*27:04:02	B*27:05:20	B*27:14	B*27:42
B*27:04:03	B*27:05:21	B*27:15	B*27:43
B*27:04:04	B*27:05:22	B*27:16	B*27:44
B*27:04:05	B*27:05:23	B*27:17	B*27:45
B*27:04:06	B*27:05:24	B*27:18	B*27:46
B*27:05:02:01	B*27:05:25	B*27:19	B*27:47
B*27:05:02:02	B*27:05:26	B*27:20	B*27:48
B*27:05:02:03	B*27:05:27	B*27:21	B*27:49
B*27:05:02:04Q	B*27:05:28	B*27:23	B*27:50:01
B*27:05:02:05	B*27:05:29	B*27:24	B*27:50:02
B*27:05:03	B*27:05:30	B*27:25	B*27:51
B*27:05:04	B*27:05:31	B*27:26	B*27:52
B*27:05:05	B*27:05:32	B*27:27	B*27:53
B*27:05:06	B*27:06	B*27:28	B*27:54
B*27:05:07	B*27:07:01	B*27:29	B*27:55
B*27:05:08	B*27:07:02	B*27:30	B*27:56

continued on next page >

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

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GENVINSET

HLA-B27

HLA-B27 (IMGT-HLA 3.31.0) family alleles detected by GENVINSET B27

B*27:57	B*27:86	B*27:111	B*27:140
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
B*27:64N	B*27:90:04	B*27:118	B*27:147
B*27:65N	B*27:91	B*27:119	B*27:148
B*27:66N	B*27:92	B*27:120	B*27:149
B*27:67	B*27:93	B*27:121	B*27:150
B*27:68	B*27:94N	B*27:122	B*27:151
B*27:69	B*27:95	B*27:123	B*27:152
B*27:70	B*27:96:01	B*27:124	B*27:153
B*27:71	B*27:96:02	B*27:125	B*27:154
B*27:72	B*27:97	B*27:126	B*27:155
B*27:73	B*27:98	B*27:127	B*27:156
B*27:74	B*27:99	B*27:128	B*27:157
B*27:75	B*27:100	B*27:129	B*27:158
B*27:76	B*27:101	B*27:130	B*27:159
B*27:77	B*27:102	B*27:131	B*27:160
B*27:78	B*27:103	B*27:132	B*27:161
B*27:79	B*27:104	B*27:133	B*27:162
B*27:80	B*27:105	B*27:134	B*27:163
B*27:81	B*27:106	B*27:135	B*27:164
B*27:82	B*27:107	B*27:136	B*27:165
B*27:83	B*27:108	B*27:137	B*27:166
B*27:84	B*27:109	B*27:138	B*27:167
B*27:85	B*27:110	B*27:139	

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

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Limitations of the procedure

- The method detects all HLA-B27 group alleles except HLA B*27:23/ B*27:52/B*27:67/ B*27:92/ B*27:157 (IMGT-HLA 3.31.0).
- The described conditions for the PCR should be precisely controlled. Deviations from these parameters can lead to poor results.
- All GENVINSET work must be made according to general lab best practices and be adjusted to local regulations, like the EFI standard (European Federation of Immunogenetics).
- The rt-PCR thermal cycler must be calibrated according to the manufacturers' recommendations and should be used in accordance to manufacturers' 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 suspicions of possible loss of reactivity, contamination, container 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.
- Eliminate expired reagents according to applicable regulations.

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Troubleshooting guide

Problem

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

Reaction Blank (H_2O) is positive.

- **Primer Mix/Master Mix/Reaction Blank contamination**
  - Repeat the experiment with new Primer Mix/Master Mix/ Reaction Blank aliquots.
  - Perform the kit components manipulation always according to usually accepted practices to avoid contamination.
  - Verify manipulation and storage conditions.
  - Discard 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.
  - Confirm suitability of the used reagents (1.5 mL tubes, pipette tips).
- **Pipetting error**
  - Always check that the added sample matches the sample sheet.

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

- **Samples with very low DNA concentration**
  - Check the cellular lysate DNA concentration.
- **DNA samples with high concentration**
  - Perform a DNA extraction method validation assay testing some dilution of the DNA samples.

Fluorescence intensity too low

- **Kit degradation (Primer Mix or Master Mix vials)**
  - Confirm the kits correct storage (Primer Mix vial stored in darkness and Master Mix vial frozen).
  - Avoid more than 3 freeze/unfreeze cycles of the primer mix vial.
  - Aliquote the reagents if necessary.
  - Repeat the series with new reagents.

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Negative control sample (B27 neg.) is positive

- **Cross contamination**
  - Handle the kit's components always with all currently contamination avoidance practices .
- **Pipetting error**
  - Always check that the added sample matches the sample sheet.

Positive control sample (B27 pos.) is negative

- **Pipetting error**
  - Always check that the added sample matches the sample sheet.

Fluorescence intensity varies

- **There is dirt on the outside which interferes with the signal**
  - Manipulate the plates always wearing gloves.
- **The volume is not at the bottom of the well or there is an air bubble**
  - Centrifuge to make sure the sample is at the bottom of the well and there are no air bubbles, according to the technics protocol.
- **Pipetting error**
  - Verify the volume added in each well/capillary is correct.

There is no fluorescence signal

- **Incorrect reading channels selected**
  - Configure the correct reading channels.
- **Pipetting error or reagent absence**
  - Control the pipetting and the reactions configuration.
  - Repeat the PCR.
- **No reading channel was selected in the thermal cycler's program.**
  - Revise and modify the thermal cycler program.

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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.

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