GenVinSet

HLA B57

Instructions for use

Kit for HLA B*57:01 detection

For In Vitro Diagnostics Use

Reference GVSB5703-48 (48 test)

Store from –18 to –30ºC

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GenVinSet

HLA-B57

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Intended use

GENVINSET is a kit for HLA-B*57:01 allele screening by Real time PCR using Taqman® probes technology.

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Summary and explanation

Abacavir sulfate, also known 1592U89, is a synthetic carboxylic nucleoside drug, reverse transcriptase inhibitor, which is used to treat HIV and AIDS.

The drug is used to treat type 1 HIV and should always be used together with other antiretroviral agents. Abacavir should never be used as the only prospect when changing antiretroviral regimens due to loss of viral response.

This drug has been associated with the occurrence of fatal hypersensitivity reaction. Symptoms include fever, skin rash, fatigue, gastrointestinal symptoms such as nausea, vomiting, diarrhea or abdominal pain. Others are respiratory symptoms such as pharyngitis, dyspnea or cough. Hypersensitivity is associated with the HLA-B*5701, for which before starting treatment with this drug it is necessary to conduct a study of the presence of this allele in the patient to avoid such a hypersensitivity reaction.

Within the major histocompatibility complex, the HLA-B*5701 allele is a polymorphism of HLA-B and has a population frequency of around 3% in European population.

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Procedure principles

The detection method used by Genvinset HLA-B57 is based in a primer specific PCR for the HLA B*57:01 alleles (see section 'Alleles detected by GENVINSET B57', page 15) monitored with Taqman® probes.

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

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

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Kit contents

Reference GVS B5703-48 (48 tests)

- GVS B57PMv3: 2 x 110 µL Primer Mix (PM).
- GVS-B57-MM: 2 x 138 uL Master Mix (MM)
- GVS-B57-C+: 1 x 5 uL Positive Control (C+)
- GVS-RB: 1 x 100 uL Reaction Blank (RB)

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Kit storage

All of the kit reagents should be stored between -18ºC and -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 (GVS-B57PMv3) and the Master Mix (GVS-B57-MM) vials, 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, P100 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
- Vortex
- 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 emitting results with diagnostic purposes, perform a validation test of the samples obtained with the extraction system.

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.
- 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 (B57:01+) included in the kit, as well as a negative B57:01 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 optical plate and add 1 uL of DNA, or Positive Control or Reaction Blank, in the contamination control well.

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

5. Place the plate in the thermocycler and start the program.

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B) Thermal cycler configuration

1. Set up the next amplification program:

	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

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. 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 'Materials required but 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 'B)Thermal cycler configuration'). Select the step 60 sec at 64 ºC, and click "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. Click "Gain Optimization" 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 then "Add". In the window "Auto-Gain Optimization 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 Optimization Before 1st Acquisition", and click "Close".

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

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Results

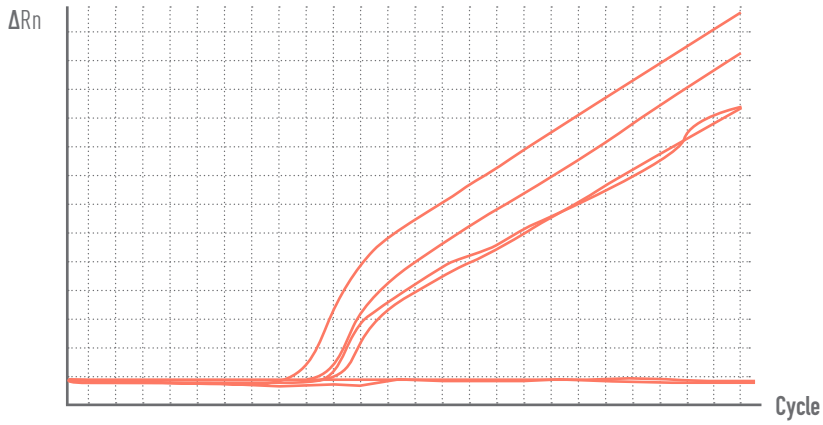
GENVINSET B57 is a qualitative technique in which we will identify presence or absence of the HLA B*57:01 alleles (See section 'Alleles detected by GENVINSET B57' page 15).

It is not necessary to select any passive reference.

The results of this technique can be obtained as follows:

B57:01 results

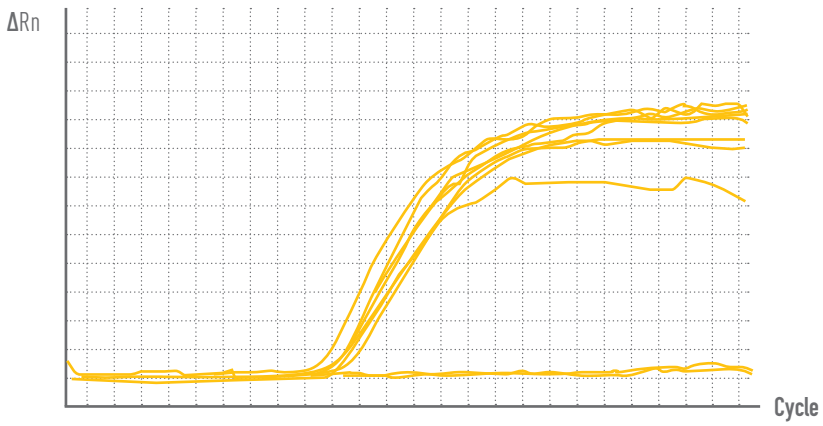
Selecting FAM channel in Amplification Plot, we can see next graphic:



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

ß-globin results

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



Samples generating a yellow amplification curve are positive for internal control (ß-globin). Curve can diminish or disappear when FAM reaction is positive.

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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-B57:01 typing).

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

- The contamination control (Reaction Blank) must provide negative results for ß-globin considering a Cp >35 as being negative. Cp values <35 will show a contamination in the session so results should be discarded.
- A positive control sample (B*57:01) must be positive for both B*57 and ß-globin. ß-globin curve can diminish or disappear when FAM reaction is positive.
- The samples should always be positive for ß-globin (Cp <35). Curve can diminish or disappear when FAM reaction is positive.
- The samples generating results with Cp >35 for ß-globin must be considered as doubtful and must be retested performing a new extraction of DNA.

The assay must be made according to the kit 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

This technique provide a high degree of resolution for each primer pair because they recognize 2 DNA areas located in *cis*, providing high sensitivity, specificity and reproducibility.

2. Analytical sensitivity

Once performed a dilution assay using 1:4 serialized dilutions of two DNA samples, one of them B*57:01 positive and the other one B*57:01 negative, obtained by a conventional extraction system, at an initial concentration of 30.4 and 29.0 ng/uL respectively, the following results were obtained as for analytical sensitivity of the B*57:01 allele detection:

- DNA sample obtained by conventional extraction system:
  Detection Limit = 0.5 ng/µL (*)

  (*) Cp < 35

3. Diagnostic sensitivity and specificity

In two studies of human genomic DNA, performed in external laboratories, 75 samples were analyzed with GENVINSET HLA B57v3 kit. They were previously typed by SSO in Luminex platform or by SBT.

Of the 75 analyzed samples all of them were validated (positive amplification of the ß -globin control gene), and 21 were positive.

	GENVINSET B57		
	Samples	B57:01+	B57:01-
SSO/ SBT	B57:01 +	21	0
	B57:01 -	0	54

There is a 100% match in the results obtained with GENVINSET B57v3 and previous information of samples.

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Alleles detected by GENVINSET B57 (IMGT-HLA 3.31.0)

B*57:01:01:01	B*57:13	B*57:48	B*57:87
B*57:01:01:02	B*57:14:01	B*57:49	B*57:88
B*57:01:02	B*57:14:02	B*57:50	B*57:89
B*57:01:03	B*57:15	B*57:51	B*57:90
B*57:01:04	B*57:16	B*57:52	B*57:91
B*57:01:05	B*57:18	B*57:54	B*57:93
B*57:01:06	B*57:19	B*57:55	B*58:01:05
B*57:01:07	B*57:20	B*57:56	B*15:171
B*57:01:08	B*57:21	B*57:58	
B*57:01:09	B*57:22	B*57:59	
B*57:01:10	B*57:23	B*57:60	
B*57:01:11	B*57:24	B*57:61	
B*57:01:12	B*57:25	B*57:62	
B*57:01:13	B*57:26	B*57:64	
B*57:01:14	B*57:27	B*57:65	
B*57:01:15	B*57:29	B*57:67:01	
B*57:01:16	B*57:30	B*57:67:02	
B*57:01:17	B*57:31	B*57:68	
B*57:01:18	B*57:33	B*57:69	
B*57:01:19	B*57:34	B*57:71	
B*57:01:20	B*57:35	B*57:72	
B*57:01:21	B*57:36	B*57:73	
B*57:01:22	B*57:37	B*57:74	
B*57:01:23	B*57:38	B*57:75	
B*57:01:24	B*57:40	B*57:77	
B*57:06	B*57:43	B*57:78	
B*57:08	B*57:44	B*57:79N	
B*57:10	B*57:45	B*57:85	
B*57:11	B*57:47	B*57:86	

Of all amplified alleles, only the disease associated B*57:01 is a frequent one. All other alleles have extremely low frequencies and are not likely to be found again in a significant number of unrelated subjets.

- 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

- Review the table in section 'Alleles detected by GENVINSET HLA B57', page 15). Apart from B*57:01, some non frequent alleles can be detected with this kit, with a very low probability.
- 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 thermocycler 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 (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.

- **Bad quality of DNA samples**
  - Repeat the DNA extraction.
- **Samples with very low DNA concentration**
  - Revise the 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 in darkness and Master Mix vial frozen).
  - Avoid more than 3 freeze/unfreeze cycles of the Primer Mix / Master Mix vials.

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

Negative control sample (B57:01 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 (B57:01 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/capillaries 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/tube 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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1. "Real-Time PCR Using Fluorescent Resonance Emission Transfer Probes for HLA-B Typing" Rosa Faner, Natàlia Casamitjana, Jordi Coll, Pepi Caro, Ricardo Pujol-Borrell, Eduard Palou and Manel Juan.

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