GenVinSet

IL28B

Instructions for Use

Kit for determination of the C/T di-allelic system of the rs12979860 microsatellite of IL28B gene

Reference GVS-IL28B -48 (48 tests)

Store from –18 to –30ºC

CE

Rv01 / 30-07-2015

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Intended Use

GENVINSET IL28B is a kit for determination of the C/T di-allelic system of the rs12979860 microsatellite of IL28B gene by Real Time PCR using Taqman probes technology.

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Summary and explanation

Hepatitis C is one of the most common liver diseases worldwide with a 170 million of a HCV-infected individuals (1); it is often complicated by the development of cirrhosis and hepatocellular carcinoma. If an HCV infection is not treated or is ineffectively treated, hepatocellular function may deteriorate progressively (2). For patients with end stage chronic liver disease, liver transplantation (LT) is currently the treatment of choice.3 Recurrent HCV infection is one of the most important graft diseases (3, 4) that can occur after LT. The course of hepatitis C in a graft is usually more severe than the course of hepatitis C in nontransplant patients (3-6). HCV-induced fibrosis in a liver graft is an important determinant of morbidity and mortality in patients after LT; the mode of presentation varies from minor symptoms in a clinically stable patient to a rapid loss of graft function leading to graft failure. In some patients, retransplantation may be indicated despite persistent antiviral treatment (5, 7, 8).

Currently, standard antiviral therapy for HCV infection involves the administration of both pegylated interferon-α (PEGIFN- α) and ribavirin (RBV) for up to 1.5 years. A sustained virological response (SVR; ie, the patient is serum-negative for HCV by polymerase chain reaction 6 months after the cessation of therapy) occurs in approximately 40% to 50% of patients with genotype 1 and in 80% of patients with genotype 2 or 3 (9-12).

Most (70–80%) HCV infections persist and about 30% of individuals with persistent infection develop chronic liver disease, including cirrhosis and hepatocellular carcinoma (13). Epidemiological, viral and host factors have been associated with the differences in HCV clearance or persistence, and studies have demonstrated that a strong host immune response against HCV favours viral clearance (14, 15).

Thus, variation in genes involved in the immune response may contribute to the ability to clear the virus. In a recent genome-wide association study, a single nucleotide polymorphism (rs12979860) 3 kilobases upstream of the IL28B gene, which encodes the type III interferon IFN-λ3, was shown to associate strongly with more than a twofold difference in response to HCV drug treatment.

The C/C genotype was associated with a 2.5 or greater rate (depending on ethnicity) of SVR compared with the T/T genotype, and the C allele was over-represented in a random multi-ethnic population as compared with the chronically infected study cohort, raising the possibility that the C allele may favour spontaneous clearance of HCV.

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Procedure principles

The detection method used by GENVINSET IL28B is based in a primer specific PCR, which anneal to specific C or T di-allelic system of the rs12979860 microsatellite monitored with Taqman probes.

This technique provides high resolution because the primers recognize specifically this microsatellite, resulting in high sensitivity, specificity and reproducibility.

See Procedure Limitations section.

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Kit contents

Reference: GVS-IL28B-48 (48 tests)

- GVS-IL28B-PM: Primer Mix (PM). 2 vials x 248 µL
- GVS-NC: Negative Control (NC). 1 vial x 100 µL

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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 vials (GVS-IL28B-PM) as this could reduce the assays sensitivity and change results.

After receiving the kit, and once the lysis solution is unfrozen, store at room temperature (15-30ºC).

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 (P1000, P200 & P20)
- RT-PCR instrumet specific reagents (in the case of using RotorGene Q, only 0.1mL tubes are allowed)

Equipment

- RT-PCR instrument
- Vórtex
- Pipettes (P1000, P200 & P20)

Reagents

- Recombinant Taq (5U/µL) with exonuclease activity 5'—› 3' (don't use HotStart enzymes)

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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 other DNA extraction systems. Before validating results with other 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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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 1.5mL autoclaved tubes
- Use gloves and lab coat at all times.
- In each session it is recommended to use one negative control (ddH2O) and heterozygous mutations sample (C/T).

1. Take the samples out of the freezer. Vortex (or use finger knocks).

2. Prepare the Taq and primer mix to n+1 samples:

	Vol. per sample (µL)
Taq (5U/µl)	0.1
Primer Mix	9

NOTE - Use any recombinant Taq (5U/µL) with exonuclease activity 5´—› 3´ (don't use HotStart enzymes)

3. Pipette 9 µL of this mix on the capillaries over the cold holder y add 1 µL of DNA at a concentration of 20-50 ng).

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 following cycle:

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B) Thermal cycler configuration

1. Set up the next amplification program:

	Cycle number	Temperature (ºC)	Time (mm:ss)	Ramp (%)	Analysis
Denaturation	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 µL, and identify the operator and the sample.

d. Click "Edit Profile" and set up the amplification program (see subsection 'Thermal cylcer 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 Optimisation" in the "Run New Wizard" dialog box to open the "Auto-Gain Optimisation Setup" window. In the scroll menu of "Channel Settings" select "Acquiring Channels" and 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".

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Results

GENVINSET IL28B is a qualitative technique to identify the C/T di-allelic system of the rs12979860 microsatellite of IL28B gene.

It is not necessary to select any passive reference.

The results of this technique can be obtained as follows:

Amplification Plot - Cuantitative Experiment

Selecting FAM channel in Amplification Plot, to see the rs12979860 C Amplification Plot. Selecting HEX (VIC) to see rs12979860 T alleles. Samples generating an amplification curve en each channel, shows the corresponding allele.

Figure 1 shows a samples amplification plot. Red curves corresponding to FAM channel and genotype C. Green curves corresponding to HEX channel and detects genotype T.

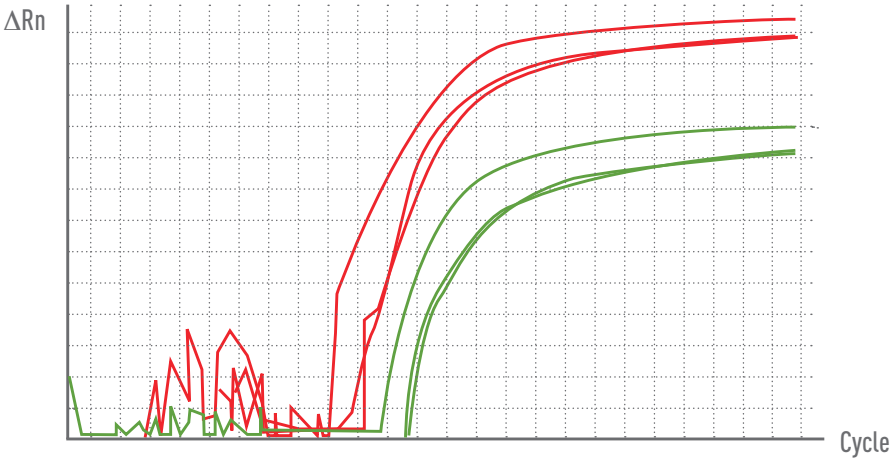

Figure 1. Two heterozygous samples (C/T), a T homozygous sample (T/T), a C homozygous sample (C/C) analyzed by rs12979860 microsatellite of IL28B gene by Real Time PCR using Taqman probes technology. Hex channel in green and Fam channel in red.

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Genotyping analysis

This genotyping analysis, selecting FAM (C) channel in Y-axis and HEX (T) channel in X-axis. The results are showed similar in figure 2.Diferents types of smaples , C/C, C/T and T/T is distributing 3 groups in the plot.Negative control is situated proximate to (0,0) point.

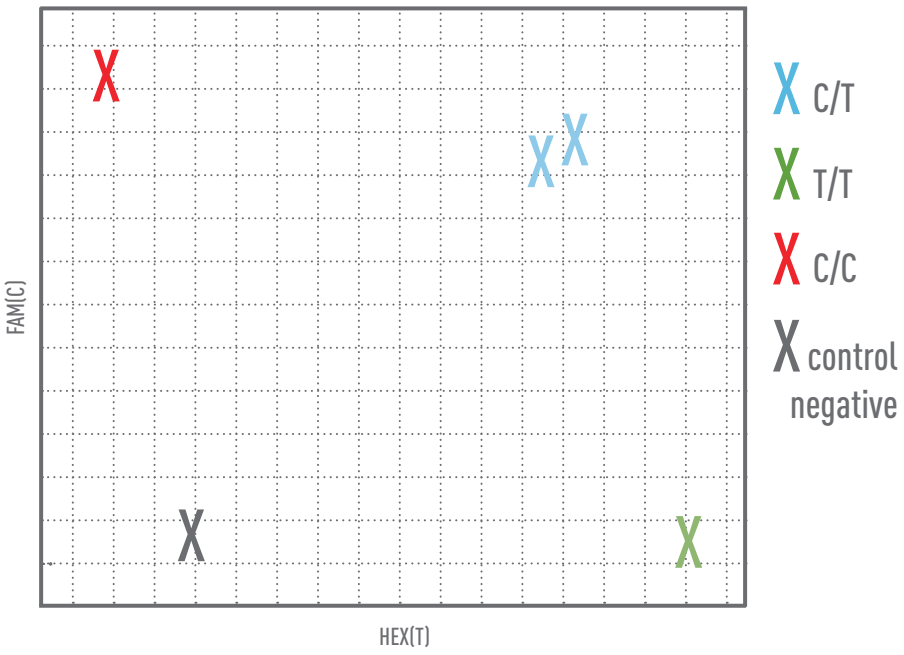

Figure 2. Two heterozygous samples (C/T), a T homozygous sample (T/T), a C homozygous sample (C/C) analyzed by rs12979860 microsatellite of IL28B gene by Real Time PCR using Taqman probes technology.

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Quality control

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

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

- The negative control (ddH20) must provide negative results both for C and T detection. 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 (heterozygous) sample must provide positive results for C and T.

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 primers of this kit are specific for human rs12979860 microsatellite of the IL28B gene. The specific amplification of these regions is ascertained at the sequencing process. There is no reported case of cross-reactivity with another gene from genomic DNA.

2. Analytical sensitivity

Once performed a dilution assay using 1:4 serialized dilutions of a DNA sample heterozygous (C/T) obtained by conventional extraction system, at a concentration of 69.4 ng/µL, the following results were obtained as for analytical sensitivity of the C/T alleles detection:

- DNA sample obtained by conventional extraction system:
  Detection Limit = 1.087 ng/µL (*)

  (*) Cp < 35

3. Diagnostic sensitivity and specificity

In a study of human genomic DNA, 35 samples obtained from a laboratory were analyzed.

	GENVINSET IL28B			
	Samples	TT	CC	CT
Real Time	TT	10	0	0
	CC	0	12	0
	CT	0	0	13

There is a 100% match in the results obtained with GENVINSET IL28B.

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Procedure limitations

- 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.
- The rt-PCR thermal cycler must be calibrated according to the manufacturers´ recommendations y 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.
- Eliminate expired reagents according to applicable regulations.

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Troubleshooting guide

Negative control (H2O) is positive

- **Primer Mix/Negative control contamination**
  - Repeat the experiment with new Primer Mix/Negative control 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)
  - Confirm there is no Taq contamination
- **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**
  - Repeat the sample extraction verifying each step (Hemoglobin can interfere with the PCR)
- **Blood processed without previous frozen step**
  - Repeat extraction with a new blood aliquot previously frozen
- **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 vial)**
  - Confirm the kits correct storage (Primer Mix vial stored in darkness)
  - Avoid more than 3 freeze/unfreeze cycles of the primer mix vial
  - Aliquot the reagent if necessary
  - Repeat the series with new reagents

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- Taq has lost activity
  - Confirm Taq's activity
  - Repeat with new Taq

Negative control sample (B27 neg.) is positive

- Cross contamination
  - Always handle the kit's components with all current 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
  - Always wear gloves when manipulating the plates
- 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 technical protocol
- Pipetting error
  - Verify the volume added in each well 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. *Centers for Disease Control and Prevention.. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR Recomm. Rep. 47, 1–39 (1998).*

2. *Iadonato SP, Katze MG. Genomics: hepatitis C virus gets personal. Nature 2009;461:357-358.*

3. *Neumann UP, Berg T, Bahra M, Puhl G, Guckelberger O, Langrehr JM, Neuhaus P. Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow-up. Transplantation 2004;77:226-231.*

4. *Berenguer M. Recurrent allograft disease: viral hepatitis. Acta Gastroenterol Belg 2005;68:337-346.*

5. *Berenguer M, Prieto M, Palau A, Rayon JM, Carrasco D, Juan FS, et al. Severe recurrent hepatitis C after liver retransplantation for hepatitis C virus-related graft cirrhosis. Liver Transpl 2003;9:228-235.*

6. *Prieto M, Berenguer M, Rimola A, Loinaz C, Barrios C, Clemente G, et al. Liver transplantation in hepatitis C. A Spanish multi-centre experience. Eur J Gastroenterol Hepatol 1998;10:771-776.*

7. *Neumann UP, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. J Hepatol 2004;41:830-836.*

8. *Burra P. Hepatitis C. Semin Liver Dis 2009;29:53-65.*

9. *Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958-965.*

10. *Fried MW, Peter J, Hoots K, Gaglio PJ, Talbut D, Davis PC, et al. Hepatitis C in adults and adolescents with hemophilia: a randomized, controlled trial of interferon alfa-2b and ribavirin. Hepatology 2002;36(4 pt 1):967-972.*

11. *Ferenci P, Scherzer TM, Kerschner H, Rutter K, Beinhardt S, Hofer H, et al. Silibinin is a potent antiviral agent in patients with chronic hepatitis C not responding to pegylated interferon/ribavirin therapy. Gastroenterology 2008;135:1561-1567.*

12. *Terrault NA, Berenguer M. Treating hepatitis C infection in liver transplant recipients. Liver Transpl 2006;12: 1192-1204.*

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13. *Seeff, L. B. Natural history of chronic hepatitis C. Hepatology 36 (suppl. 1), S35–S46 (2002).*

14. *Cooper, S. et al. Analysis of a successful immune response against hepatitis C virus. Immunity 10, 439–449 (1999).*

15. *Rehermann, B. & Nascimbeni, M. Immunology of hepatitis B virus and hepatitis C virus infection. Nature Rev. Immunol. 5, 215–229 (2005).*

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