GenVinSet

HLA-A29

Instructions for Use

Kit for HLA-A29 group of alleles detection

Reference GVS-A29-48 (48 test)
GVS-A29-24 (24 tests)

Store from –18 to –30ºC

For In Vitro Diagnostic Use CE 0086

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Intended use

GENVINSET HLA-A29 is a kit for the determination of the HLA-29 group of alleles, by Real Time PCR using Taqman® probes technology.

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Summary and explanation

Birdshot retinochoroidopathy (BSRC) is a rare, chronic, bilateral, posterior uveitis characterized by distinctive, multiple, hypopigmented choroidal and retinal lesions, first identified by Ryan and Maumenee [1]. Blurred vision and floaters are the most prevalent visual symptoms. Patients may also report dyschromatopsia and poor contrast sensitivity. Macular edema or atrophy is the most common cause of decline in visual acuity and 10% of patients are legally blind. Middle aged Caucasians of northern European extraction are most commonly afflicted [2-5]. The pathogenesis is unknown, but HLA-A29 positivity appears to confer predisposition [6-10], and retinal autoimmunity seems to play a role [9]. Vascular disease [4], sarcoidosis [4, 11], psoriasis [12], autoimmune sensorineural hearing loss [13], and vitiligo [2] have been reported in birdshot patients.

The relative risk of BSRC among HLA-A29-positive individuals has been estimated to be 50 to 224, and most investigators recognize the presence of the HLA-A29 allele as a necessary criterion for BSRC diagnosis for research purposes. HLA-A29 is present in as many as 7% of Caucasians, and is subdivided into more than 20 subtypes—mostly A*2902 in Caucasians and A*2901 in Asians. Even Asians who live in the same country as Caucasians in Europe or in the United States appear to be exempt from BSRC. Moreover, African-Americans living in North America and carrying A*2902 at a frequency of 3.57% appear to be immune from the disease. We found that gene sequences from all patients and healthy individuals sharing the A*2901 or A*2902 subtype were identical [14-16]. Furthermore, the study of polymorphisms in the HLA-A region in patients and control subjects showed no differences but surprisingly defined two strong A*2901 and A*2902 extended haplotypes [16].

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Procedure principles

The detection method used by Genvinset is based on a primer specific PCR, which anneals to specific HLA-A29 (*) group of alleles 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, high sensitivity, specificity and reproducibility.

(*) See section 'Procedure limitations' (page 16).

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Kit contents

Reference GVS-A29-48 (48 tests)

- GVS-A29-PM: 2 vials x 248 µL Primer Mix (PM)
- GVS-NC: 1 vial x 100 µL Negative Control (NC)

Reference GVS-A29-24 (24 tests)

- GVS-A29-PM: 1 vial x 248 µL Primer Mix (PM)
- GVS-NC: 1 vial x 100 µL Negative Control (NC)

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Kit storage

All of the kit's reagents should be stored from -18ºC to -30ºC, they are stable at this temperature until the expiration date, as indicated on the bottle. Do not perform more than 3 freezing/thawing cycles to the Primer Mix vials (GVS-A29-PM) as this could reduce the assays sensitivity and change results.

Due to the reagent's photo sensitive 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)
- 1.5 mL autoclaved tubes
- RT-PCR instrument 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 5' —› 3' exonuclease activity

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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 sample for HLA-A29 and one positive sample (A29).

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´

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

4. Seal the plate with convenient sealer, spin down the volume by centrifuging for 1 minute at 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
		60	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 60 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".

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

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Results

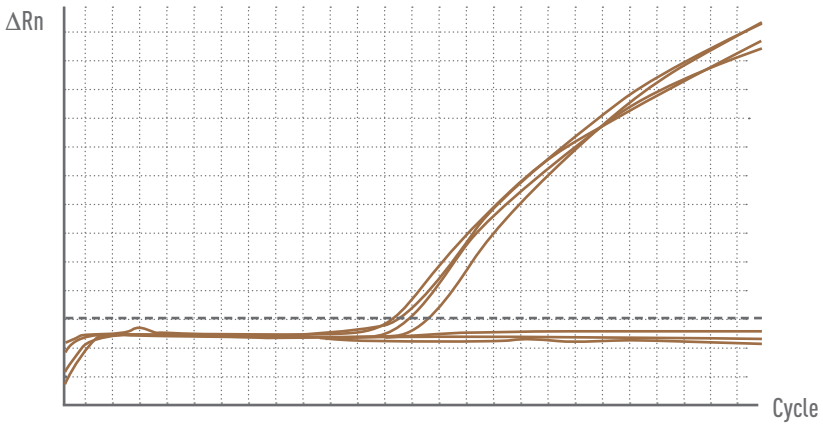
GENVINSET HLA A29 is a qualitative technique to identify presence or absence of the HLA-A*29 group of alleles.

It is not necessary to select any passive reference.

The results of this technique can be obtained as follows:

HLA-A29 results

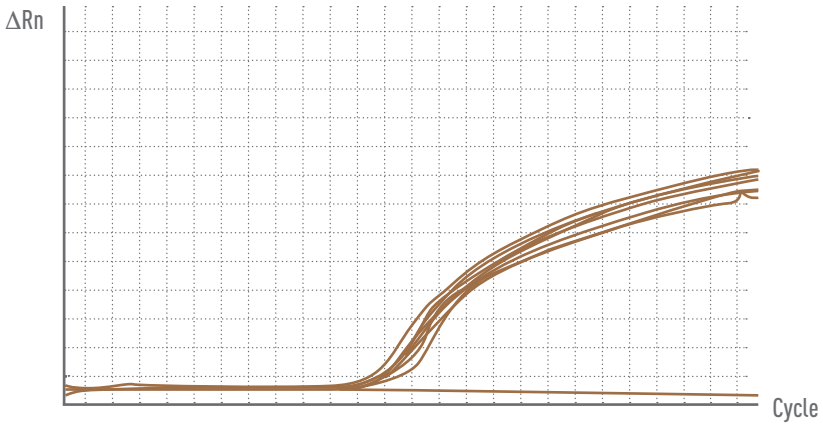
Selecting FAM channel in Amplification Plot, we can see the next graphic:



Samples generating an amplification curve are A29 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 the 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 carry out a contamination control by replacing the DNA sample for the Negative Control provided with the kit, and to introduce an A29 positive control sample.

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

- Negative control (NC) must provide negative results both for A29 and for ß-globin. Cp (Crossing Point) values >35 should be considered as a 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 A29 and for ß-globin.
- Every DNA sample should be positive for ß-globin.
- DNA samples generating results with Cp >35 should be considered as doubtful and must be retested by performing a new DNA extraction.

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.

Specificity of the analysis reactions are detailed at section 'HLA-A29 family alelles (IMGT-HLA 3.28.0) detected by GENVINSET HLA A29', page 15.

2. Analytical sensitivity

A dilution assay was performed using 1:4 serialized dilutions of one positive A*29 DNA sample and one negative A*29 DNA sample, obtained by a conventional extraction system, at a concentration of 63 and 83 ng/µL. The following results were obtained for analytical sensitivity of the A*29 allele detection:

- DNA sample obtained by conventional extraction system:
  Detection Limit = 0.32 ng/µL (*)

  (*) Cp < 35

3. Diagnostic sensitivity and specificity

In two studies of human genomic DNA, 90 samples obtained from two clinical laboratories were analyzed. They were previously typed by HLA-SSO in Luminex platform or SSP+serology.

Out of the 90 samples tested, all of them were validated (positive amplification of the ß-globin control gene), and 60 of them were found to be A29 positive:

	GENVINSET		
SSO / SSP Serology	Samples	A29 +	A29 -
	A29 +	60	0
	A29 +	0	30

There is a 100% match in the results obtained with GENVINSET HLA A29 and the typing previously obtained with the SSO (Sequence-Specific Oligonucleotide typing), SSP (Sequence-Specific primed typing) or serology methodologies.

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HLA A29 family alleles (IMGT-HLA 3.28.0) detected by GENVINSET HLA-A29

A*29:01:01:01	A*29:02:20	A*29:34	A*29:67
A*29:01:01:02N	A*29:03	A*29:35	A*29:68
A*29:01:02	A*29:04	A*29:36	A*29:69
A*29:01:03	A*29:05	A*29:37	A*29:70
A*29:01:04	A*29:06	A*29:38	A*29:71
A*29:01:05	A*29:07	A*29:39	A*29:72
A*29:01:06	A*29:08N	A*29:40	A*29:73
A*29:01:07	A*29:09	A*29:41	A*29:74
A*29:01:08	A*29:10:01	A*29:42	A*29:75
A*29:01:09	A*29:10:02	A*29:43	A*29:76
A*29:02:01:01	A*29:11	A*29:44	A*29:77
A*29:02:01:02	A*29:12	A*29:45	A*29:78N
A*29:02:01:03	A*29:13	A*29:46	A*29:79
A*29:02:01:04	A*29:14	A*29:47	A*29:80
A*29:02:02	A*29:15	A*29:48	A*29:81
A*29:02:03	A*29:16	A*29:49	A*29:82
A*29:02:04	A*29:17	A*29:50	A*29:83
A*29:02:05	A*29:18	A*29:51	A*29:84
A*29:02:06	A*29:19	A*29:52	A*29:85
A*29:02:07	A*29:20	A*29:53	A*29:86
A*29:02:08	A*29:21	A*29:54	A*29:87
A*29:02:09	A*29:22	A*29:55	A*29:88
A*29:02:10	A*29:23	A*29:56	A*29:89
A*29:02:11	A*29:24	A*29:57	A*29:90
A*29:02:12	A*29:25	A*29:58	A*29:91
A*29:02:13	A*29:26	A*29:59	A*29:92
A*29:02:14	A*29:27	A*29:60	A*29:93
A*29:02:15	A*29:28	A*29:61	A*29:94
A*29:02:16	A*29:29	A*29:62	A*29:95
A*29:02:17:01_ _ _	A*29:30	A*29:63	A*29:96
A*29:02:17:02	A*29:31	A*29:64	A*29:97
A*29:02:18	A*29:32	A*29:65	A*29:98
A*29:02:19	A*29:33	A*29:66	A*29:99

• Detected allele.

• 1 mismatch (MM) within the region comprised for the rt-PCR from the Genvinset HLA-A29 kit but they are not tested.

• more than 1 MM within the region comprised for the rt-PCR from the Genvinset HLA-A29 kit but they are not tested.

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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 laboratory 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 manufacturer´s recommendations and should be used in accordance to 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 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

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 DNA concentration and repeat extraction
- **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)

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- Avoid more than 3 freeze/unfreeze cycles of the primer mix vial
- Aliquote the reagents if necessary
- Repeat the series with new reagents

• **Taq has lost activity**

- Confirm Taq's activity
- Repeat with new Taq

Negative control sample (A29 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 (A29 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. Ryan SJ, Maumenee AE. Birdshot retinochoroidopathy. Am J Ophthalmol. 1980;89:31–45.

2. Gass JD. Vitiliginous chorioretinitis. Arch Ophthalmol 1981;99:1778–87.

3. Kaplan HJ, Aaberg TM. Birdshot retinochoroidopathy. Am J Ophthalmol 1980;90:773–82.

4. Priem HA, Oosterhuis JA. Birdshot chorioretinopathy: clinical characteristics and evolution. Br J Ophthalmol 1988;72:646–59.

5. Ryan SJ, Maumenee AE. Birdshot retinochoroidopathy. Am J Ophthalmol 1980;89:31–45.

6. Baarsma GS, Kijlstra A, Oosterhuis JA, et al. Association of birdshot retinochoroidopathy and HLA-A29 antigen. Doc Ophthalmol 1986;61:267–9.

7. Baarsma GS, Priem HA, Kijlstra A. Association of birdshot retinochoroidopathy and HLA-A29 antigen. Curr Eye Res 1990;9:63–8.

8. LeHoang P, Ozdemir N, Benhamou A, et al. HLA-A29.2 subtype associated with birdshot retinochoroidopathy. Am J Ophthalmol 1992;113:33–5.

9. Nussenblatt RB, Mittal KK, Ryan S, et al. Birdshot retinochoroidopathy associated with HLA-A29 antigen and immune responsiveness to retinal S-antigen. Am J Ophthalmol 1982; 94:147–58.

10. Priem HA, Kijlstra A, Noens L, et al. HLA typing in birdshot chorioretinopathy. Am J Ophthalmol 1988;105:182–5.

11. Yoshioka T, Yoshioka H, Tanaka F. Birdshot retinochoroidopathy as a new ocular sign of sarcoidosis. Nippon Ganka Gakkai Zasshi 1983;87:283–8.

12. Hesse S, Berbis P, Chemila JF, et al. Psoriasis and birdshot chorioretinopathy: response to aromatic retinoids. Dermatology 1993;187:137–9.

13. Heaton JM, Mills RP. Sensorineural hearing loss associated with birdshot retinochoroidopathy. Arch Otolaryngol Head Neck Surg 1993;119:680–1.

14. Tabary T, Prochnicka-Chalufour A, Cornillet P, Lehoang P, Betuel H, Cohen JHM. HLA-A29 sub-types and "Birdshot" choroido-retinopathy susceptibility: a possible "resistance motif" in the HLAA29.1 molecule (in French). C R Acad Sci Paris. 1991;313: 599–605.

15. LeHoang P, Ozdemir N, Benhamou A et al. HLA-A29.2 subtype associated with birdshot retinochoroidopathy. Am J Ophthalmol. 1992;113:33–35.

16. Donvito B, Monnet D, Tabary T, et al. Different HLA classI A region complotypes for HLA-A29.2 and -A29.1 antigens, identical in birdshot retinochoroidopathy patients or healthy individuals. Invest Ophthalmol Vis Sci. 2005;46:3227–3232.

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