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EliGene® Spondylitis HLA-B27 C6

REF 90060-C6 **(for 192 samples)**

Kit components:

1 x 580 µl **HLA-B27 Mix**
1 x 150 µl **PC DNA HLA-B27**
Instruction for Use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20°C. Kit and remaining components must be stored at -20°C in darkness.

Intended use

EliGene® Spondylitis HLA-B27 C6 is intended for detection of HLA-B27 allele (subtypes B*2701-2759) from isolated DNA.

Principle of the method

This diagnostic kit is based on RealTime PCR method. In this kit primers and labeled probes (FAM and HEX) for the detection of HLA-B27 alleles and for the detection of internal control are used.

Introduction

Human Leukocyte Antigen (HLA) B27 (subtypes B*2701-2759) is an HLA class I surface antigen that is encoded in the B locus in the major histocompatibility complex (MHC) on the short arm of chromosome 6. In HLA-B27 antigen a strong association with ankylosing spondylitis (AS) and spondyloarthropathies (SpA) was found. Moreover the increased incidence of this antigen in other diseases as Reiter´s syndrome or uveitis was found. Association studies found an association between HLA-B27 antigen and AS in all ethnic and racial groups worldwide however the prevalence of HLA-B27 antigen and the strength of its association with AS does vary. For example, the prevalence of HLA-B27 antigen is about 8% in Caucasians, 4% in North Africans, 2-9% in Chinese, and 0.1-0.5% in Japanese. Further, among northern Europeans only 8% of the general population possesses HLA-B27, but more than 90% of the patients with AS possess this gene. In contrast, among African Americans 2% to 4% of the general population and only 50% to 60% of patients with AS possess this gene.

From this reasons HLA-B27 test cannot be used to screen an asymptomatic population to detect AS but the test provides a statement of increased probability of the existence of AS in the symptomatic patient. Also, the presence of HLA-B27 antigen influences the clinical manifestations of AS disease, because HLA-B27–positive patients have a significantly younger age at onset of their disease and a higher prevalence of episodes of eye inflammation (acute anterior uveitis) and hip joint involvement.

EliGene® Spondylitis HLA-B27 C6 kit detects HLA-B27 allele (subtypes B*2701-2759). As an internal control the gene SYPL2 (synaptophysin-like 2) is used.

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Primary sample collection, handling and storage

Clinical material:	Recommended DNA isolation procedure:
Blood	Automatic: cobas® 6800/8800 Systems (Roche)

Blood:

Automatic isolation:

Isolate DNA from 200 µl of whole blood 3, 5 or 7 times diluted with PBS buffer or physiologic water to the minimal volume 350 µl with using cobas® 6800/8800 Systems (Roche) according to the protocol. Dilution does not affect the quality of the result.

Additional required equipment

- Repeater pipette, automated pipette and sterile tips (1 mL and 10 mL) – DNA, RNA free, DNase-, RNase-free (we recommended plastic with CE certificate for diagnostic purposes).
- PBS buffer or physiologic water.
- Equipment for RealTime PCR – the kit is designed for **cobas® 6800/8800 Systems (Roche)**.
  - cobas omni Utility Channel Reagent kit containing Utility Channel Master Mix Reagent 2 (UC MMx-R2) and Utility Channel Reagent Cassete with Protease, internal control, elution buffer, Master Mix Reagent 1 (MMx-R1) and empty container for prepared MasterMix Reagent 2.
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For HLA-B27 allele the probe labeled with FAM is used (exc. 494 nm – em. 518 nm) – Channel 2
- For *SYPL2* gene (internal control) detection the probe labeled with HEX is used (exc. 520 nm – em. 548 nm) – Channel 3

cobas® 6800/8800 Systems (Roche):

1. On the “Utility channel tool” panel (UC), create a “UC analysis package” or open an existing unpublished one.

On the “UC analysis package” panel, update UC analysis package name and/or reagent cassette ID information if necessary.

2. Choose the "PCR profile" button on the "UC analysis package" and set up the following temperature profile:

Pre-PCR step

55°C	120 s	
94°C	5 s	
1st Measurement – 5 cycles		
95°C	5 s	
62°C	30 s	*Data collection
2nd Measurement – 45 cycles		
91°C	5 s	
62°C	20 s	*Data collection

3. Choose the "Parameter" button to define or edit the following parameters: sample material, processing volume, target channel names and target calling sensitivity.
   In the "Pipetting profiles group" box, choose "U_Simple sample" as a "Sample material" and 200 µl in the drop-down list of the "Processing volume". "Add" the profile.
4. In the "Channel" group box, select Channel 2 and Channel 3 and fill in the "Target name" fields and the "Sensitivity (RFI min)" fields following information:
   Channel 2 for HLA-B27 target, RFI (min)= 2.5
   Channel 3 for IC (SYPL2) target, RFI (min)= 2.5
5. Choose either the "Save as" button or the "Save" button.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mixed on vortex and shortly spun.

Preparation of Reaction Mix

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Take one microtube with HLA-B27 Mix and after the thawing pipette **564 µl** of **HLA-B27 Mix** to a new tube with **9,4 ml** of **UC MMx-R2**. Thereafter pipette **9,96 ml** of **this master mix** into the row 2 of the Reaction cassette. For detailed instructions please see guides for cobas® omni Utility Channel Reagent Kit and cobas® omni Utility Channel CE-IVD (Roche) from manufacturer of the cobas® 6800/8800 Systems (Roche).

Detection: Prepare minimal 350 µl of diluted whole blood for analysis and analyze according to the protocol mentioned above.

Positive control: Take microtube with Positive Control PC DNA HLA-B27 and after the thawing mix 10 µl of Positive Control with 400 µl of physiologic water or PBS buffer and analyze the same way as a whole blood sample.

Insert the Reaction cassette and samples to cobas® 6800/8800 System (Roche) and run the program according to chapter "Configuration of Real Time instrument" above.

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IMPORTANT NOTICE: After mixing HLA-B27 Mix together with UC MMx-R2 and loading into the Reaction cassette the Reaction cassette with master mix must be stored at 2 – 8 °C in an external fridge or in the fridge of the cobas® 6800/8800 System (Roche). Subject to these storage conditions, the master mix is stable for 1 month. Generally, prefilled Reaction cassette with master mix can be used 10 times and 30 days after the first use or before expiry dates of the components.

The Positive Control PC DNA HLA-B27 is stable for 10 freezing/thawing cycles. It should be stored at -20 °C.

Result reading

cobas® 6800/8800 Systems (Roche)

The results for a utility channel test can be viewed the same way as other test results on the cobas® 6800/8800 Systems (Roche). The sample results are identified with the UC analysis package name, and the results can be found in the "Routine" -> "Test results" panel or "Control batch" panel.

Positive result for HLA-B27 allele: The positive result is characterized by amplification and growth of signal in Channel 2 (FAM) specific for *HLA-B27 allele*. The "RFI min." value must be set to 2.5 (Sensitivity). In a case of negative results the amplification (ct value) will not occur.

The Internal Control must be amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in Channel 3 (HEX) specific for human *SYPL2 gene*. The "RFI min." value must be set to 2.5 (Sensitivity) for Channel 3.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM channel of HLA-B27 Mix does not appear before cycle number 35, the result of test should be interpreted as HLA-B27 negative. The signal for Internal Control must be positive – see article Control procedure.

Positive result:

Amplification signal in FAM channel of HLA-B27 Mix appears before cycle number 35. HLA-B27 allele was detected in the sample. The sample is HLA-B27 allele positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

If in both channels, FAM and HEX, the amplification signal of HLA-B27 Mix is not observed. In this case it is necessary to repeat the analysis. The best way is to use DNA sample prepared by new DNA isolation from fresh sample.

Control procedure

EliGene® Spondylitis HLA-B27 C6 kit detects as an internal isolation control the human gene *SYPL2* (synaptophysin-like 2) in HEX channel. *SYPL2* gene is present in each human DNA sample, so it is not necessary to add internal control to the sample. Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is HLA-B27 negative, the Ct of internal control must be Ct < 35.

Reference material:

To monitor the whole examination process covering DNA isolation and RealTime PCR detection it is possible to use reference material positive for HLA-B27 allele. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of DNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

EliGene® Spondylitis HLA-B27 C6 kit is specific for detection of HLA-B27 allele. The kit detects human genomic DNA.

Within the frame of testing the functional characteristics of EliGene® Spondylitis HLA-B27 C6 kit, totally 166 clinical specimens were analyzed. The EliGene® Spondylitis HLA-B27 C6 kit diagnosed as HLA-B27 positive all 124 specimens. Totally 42 specimens were rightly determined by EliGene® Spondylitis HLA-B27 C6 kit as HLA-B27 negative.

The calculation of sensitivity and specificity of EliGene® Spondylitis HLA-B27 C6 kit is shown in Table 1. The sensitivity and specificity of EliGene® Spondylitis HLA-B27 C6 kit is 100%.
Analytical sensitivity is 1 ng of DNA in reaction mix.
Analytical specificity of method is 100%. Analytical specificity of the method was validated by searching in the DNA databases.
Clinical specificity EliGene® Spondylitis HLA-B27 C6 kit specificity was tested on 166 samples of human DNA isolated from whole blood.

Table 1. Calculation of sensitivity and specificity of EliGene® Spondylitis HLA-B27 C6 kit

A = 124 Really positive	B = 0 False positive
C = 0 False negative	D = 42 Really negative

Sensitivity = A/(A+C) = 124/(124+0) = 100%
Specificity = D/(D+B) = 42/(42+0) = 100%

Diagnostic performance characteristics:

EliGene® Spondylitis HLA-B27 C6 kit specificity was tested on 166 samples of human DNA isolated from whole blood. Totally 166 samples were right determined by EliGene® Spondylitis HLA-B27 C6 kit.
The clinical specificity of EliGene® Spondylitis HLA-B27 C6 kit is 100%.

Measuring interval

The kit enables the detection of ≥1 ng DNA molecules in reaction mix.

Internal control of quality

As an internal control of quality the Internal Control (*SYPL2* gene) for checking the process of DNA isolation together with Positive Control for functional control of master mix and as a reference sample are used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of DNA). It is strictly recommended to use procedures and chemistry mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

The tube containing mix (HLA-B27 Mix) is disposable and is stable at -20 °C before expiry date. Do not freeze tube with HLA-B27 Mix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.

- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tube containing mix (HLA-B27 Mix) is disposable and therefore must be used once only in the preparation of the reaction mixture.
- Reagents carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.

Literature

Khan MA. Remarkable Polymorphism of HLA-B27: An Ongoing Saga. Curr Rheumatol Report. 2010; 12: 337-41

Robinson PC, Brown MA. 2012. The genetics of ankylosing spondylitis and axial spondyloarthritis. Rheum Dis Clin North Am. 38(3):539-53

Thomas GP, Brown MA. Genetics and Genomics of Ankylosing Spondylitis. Immunol Rev. 2010; 233:162-180.

Symbols

	Catalog number
	Upper limit of temperature
	Batch code
	Use by (last day of month)
	*in vitro* diagnostic medical device
	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Attention, consult instructions for use
	Manufacturer

Manufacturer

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