RIDA®GENE HLA-B27

REF PY0205

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1. Intended use

For *in vitro* diagnostic use. With the RIDA®GENE HLA-B27 kit HLA-B27 alleles in genomic DNA, isolated from human whole blood EDTA samples, are qualitatively detected by using real-time Polyermase Chain Reaction (PCR). The RIDA®GENE HLA-B27 kit is designed to assist in the diagnosis of patients with suspected spondylitis ankylosans (Bechterew's disease) and other autoimmune diseases. **The test is not to be used for tissue typing.**

The following HLA-B27 subtypes will be detected theoretically (*in silico*) with the sequence-specific primers: HLA-B*27:01 to 21, 23 to 152 and 154 to 164. Of these, the following subtypes were detected *in vitro*: HLA-B*27:01 to 05, 08 to 10, 12, 14, 23 and 26.

2. Summary and explanation of the test

The human leukocyte antigens B27 (HLA-B27) is a major class I histocompatibility complex cell surface antigen and is coded on chromosome 6. Its task is to present microbial antigens to T-cells. Almost all nucleated cells in the body have class I HLA molecules.[1]
An association with specific inflammatory, rheumatic diseases, spondyloarthritis (SpA), particularly ankylosing spondylitis (AS) is a given in carriers of HLA-B27 alleles.[2,3] The association in the Caucasian population is particularly pronounced with a 90 - 95 % prevalence of HLA-B27 in AS patients.[4,5] The prevalence of HLA-B27 in the total population varies significantly between the ethnic groups.[6] AS is a chronic, rheumatic inflammation, which mainly affects the spine and the sacroiliac joints. Other rheumatic diseases associated with HLA-B27 include Reiter's syndrome, acute anterior uveitis and inflammatory intestinal disease.[7]

The pathogenic mechanism, in which HLA-B27 causes an increased susceptibility towards the development of arthritic disease, is still unknown despite intensive research work.

3. Test principle

With the RIDA®GENE HLA-B27 kit HLA-B27 alleles in genomic DNA, isolated from human whole blood EDTA samples, are qualitatively detected by using real-time Polyermase Chain Reaction (PCR).
After the DNA isolation, the specific gene fragment and a human gene sequence (IC) as a reference gene (if available) are amplified.
The amplified target sequences are detected using the hydrolysis probes attached to one end with the quencher and a reporter fluorescence label (fluorophore) on the other end. The probes hybridize with the amplicon in the presence of a target sequence. During extension, the Taq-polymerase separates the reporter from the

quencher. The reporter emits a fluorescence signal that is detected by the optical unit of a real-time PCR device. The fluorescence signal increases with the quantity of formed amplicons.

4. Reagents provided

Tab. 1: Reagents provided (The reagents in a kit are sufficient for 100 determinations.)

Kit code	Reagents	Quantity		Cap color
1	Reaction mix	2x	1050 µl	yellow
2	Taq polymerase	1x	80 µl	red
N	No template control	1x	450 µl	white
P	Positive control	1x	200 µl	blue

5. Storage instructions

- All reagents must be stored away from light at -20 °C and can be used until the expiry date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g. in a refrigerator at 2 - 8 °C).
- Repeated freezing/thawing of up to 20 times does not have an impact on the test property (if necessary, create aliquots after the first thaw and freeze reagents immediately).
- Cool all reagents during PCR preparation (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE HLA-B27 real-time PCR test can be used with the following extraction buffers and real-time PCR devices:

Tab. 2: Validated equipment

Extraction buffer	
Promega	Maxwell® RSC
Real-time PCR device:	
Roche	LightCycler® 480II cobas z 480 Analyzer
Agilent Technologies	Mx3005P
Bio-Rad	CFX96™

If you want to use other extraction plattforms or real-time PCR instruments, please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) when using LightCycler® 480II and cobas z 480 Analyzer
- Real-time PCR consumables (plates, reaction vials, foils)
- Centrifuge with rotor for reaction vial or plates
- Vortex blender
- Pipettes (0.5 - 20 µl, 20 - 200 µl, 100 - 1000 µl)
- Pipette tips with filters
- Powder-free disposable gloves

7. Precautions for users

For *in vitro* diagnostic use.
Only trained laboratory personnel may perform this test. Follow the guidelines for working in medical laboratories. The instructions for use for performing this test must be strictly followed. Do not pipette samples or reagents using your mouth. Avoid contact with broken skin or mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are handled.

- Please ensure that the extraction, PCR solution and PCR are spatially separate in order to avoid cross-contaminations.
- Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
- Dispose of test kit once the expiry date has lapsed.

Users are responsible for proper disposal of all reagents and materials after use. Follow the respective national disposal regulations.

For further details, see the safety data sheets (SDS) www.r-biopharm.com.

8. Collection and storage of samples

8.1 Storage of samples

This test was developed for the examination of human whole blood EDTA samples. The samples must be stored at room temperature for up to 24 hours and 2 - 8 °C for up to 72 hours until the DNA is extracted.[8] A microbial contamination of the samples must be avoided. The use of heat-inactivated, lipemic, hemolytic, icteric, or cloudy samples can lead to false results.

8.2 Preparation of samples

8.2.1 DNA isolation and whole blood EDTA

A commercially available DNA isolation kit or DNA extraction system (e.g. Maxwell® RSC Instrument (Promega)) is recommended for the isolation of DNA from whole blood EDTA. The manufacturer's instructions must be observed.

When using Maxwell® RCS instruments (Promega), you are recommended to mix the blood samples for at least 5 minutes at room temperature. 30 µl proteinase K must be added to a 1.5 ml reaction vial for the preparation of samples. 200 µl from the blood sample and 300 µl from the lysis buffer must also added. Vortex the solution for 10 seconds and incubate at 56 °C for 20 minutes. 100 µl of the elution buffer must be used for the extraction. The manufacturer's further instructions must be observed.

9. Test procedure

9.1 Master-Mix preparation

The overall number of the reactions (samples and control reactions) must be calculated for the PCR.

Adding an additional 10% volume to the master mix is recommended in order to balance out the pipette loss (see Tab. 3). Prior to use, thaw, mix and briefly centrifuge the Reaction Mix, the Taq Polymerase, the No Template Control and the Positive Control. Always cool all reagents during work steps (2 - 8 °C).

Tab. 3: Example for the calculation and production of the master mix for 10 reactions

Kit code	Components of the master mix	Quantity per reaction	10 reactions (plus 10 %)
1	Reaction mix	19.3 µl	212.3 µl
2	Taq polymerase	0.7 µl	7.7 µl
	Overall	**20.0 µl**	**220.0 µl**

Mix master mix and finally, centrifuge for short time.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the master mix in to the respective reaction vials (vials/plates).

No template control: Add 5 µl of the No Template Control to the prepared master mix.

Samples: Add 5 µl DNA extract to the prepared master mix of the sample reactions.

Positive control: Add 5 µl Positive Control for the prepared master mix into the reaction vial provided.

Close the reaction vials or plates, briefly centrifuge and transfer into the real-time PCR device. Start PCR according to device settings (See Tab. 4, Tab. 5, Tab. 6, Tab. 7).

9.3 PCR Instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 4: DNA real-time PCR profile for LightCycler® 480II and cobas z 480 Analyzer

Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and extension take place in the same step.

Tab. 5: DNA real-time PCR profile for Mx3005P and CFX96™

Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: **Annealing and extension take place in the same step.**

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.

Tab. 6: Universal DNA real-time PCR profile for LightCycler® 480II and cobas z 480 Analyzer

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: **Annealing and extension take place in the same step.**

Tab. 7: Universal DNA real-time PCR profile for Mx3005P and CFX96™

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: **Annealing and extension take place in the same step.**

9.4 Detection channel set-up

Tab. 8: Selecting suitable detection channels

Real-time PCR device	Record	Detection channel	Comment
Roche LightCycler® 480II	HLA-B27	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	IC	533/580	
Roche cobas z 480 Analyzer	HLA-B27	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	IC	540/580	
Agilent Techn. Mx3005P	HLA-B27	FAM	**-**
	IC	HEX	
Bio-Rad CFX96™	HLA-B27	FAM	**-**
	IC	HEX	

10. Quality control

Specimens are evaluated using the analysis software of the respective real-time PCR device according to the manufacturer's instructions. No Template Control and Positive Control must be listed for each PCR run and must have correct results (see Tab. 9).

Tab. 9: A valid PCR run must meet the following conditions:

Sample	Results		Target gene Ct
	HLA-B27	IC	
Positive control	Positive	Positive	See Quality Assurance Certificate
No Template Control	Negative	Negative	0

If one of the two controls, No Template Control or Positive Control, is not in line with the specifications, the whole PCR run must be repeated.

The Positive Control contains a synthetic template of a gene sequence HLA-B27 and a human gene sequence IC. The proof of the Positive Control and the human samples must therefore be positive in the detection channel IC.

If the specified values are not met, check the following before repeating the test:

- Expiration date of the reagents used
- Functional performance of the equipment used (e.g., calibration)
- Correct test procedure

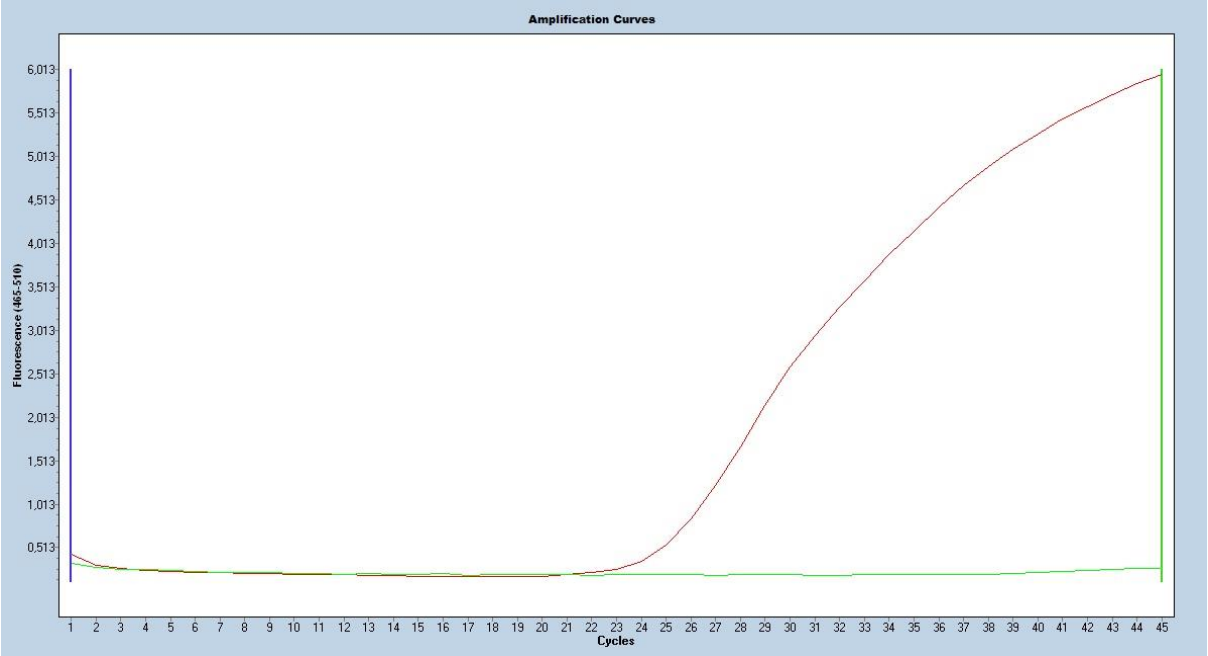

Fig. 1: Correct performance of the Positive Control and No Template Control on the cobas z 480 Analyzer (detection channel 465/510)

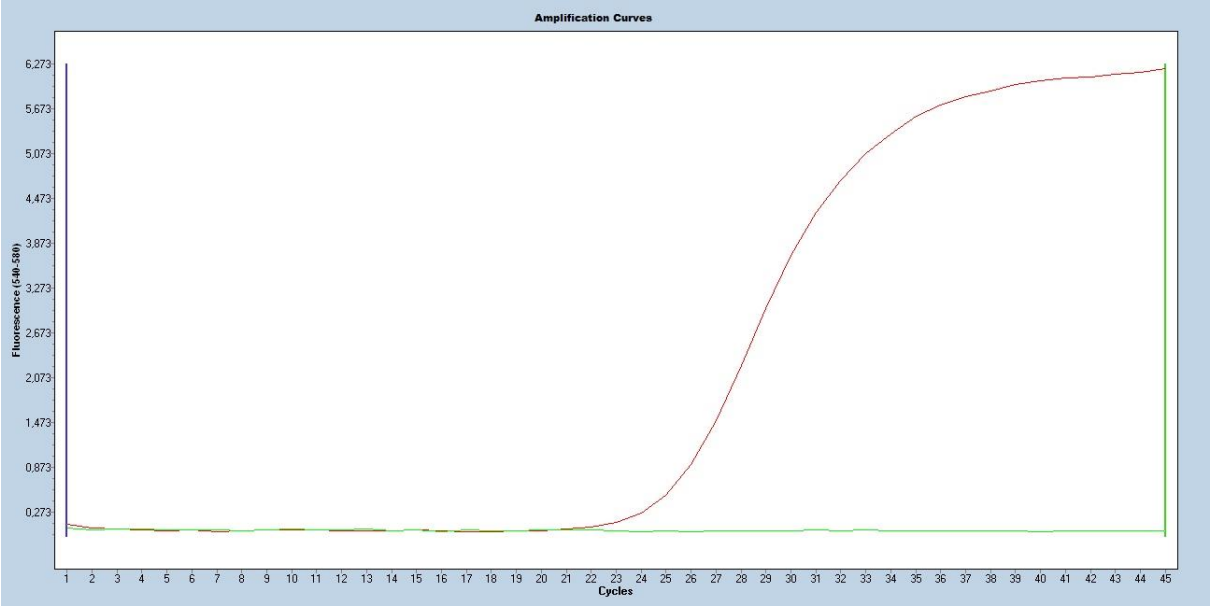

Fig. 2: Correct performance of the Positive Control and No Template Control on the cobas z 480 Analyzer (detection channel 540/580)

11. Interpretation of the Results

The sample evaluation of the results takes place as per table 10.

Tab. 10: Interpretation of the results (e.g. LightCycler® 480II)

Record	HLA-B27	IC	Results
Example sample 1	**Positive**	**Positive**	HLA-B27 positive
Example sample 2	Negative	**Positive**	HLA-B27 negative
Example sample 3	**Positive**	Negative	Invalid
Example sample 4	Negative	Negative	Invalid

The PCR run cannot be evaluated, if the Positive Control displays no amplification in the detection system. The whole PCR run must be repeated.

If the Positive Control in the detection system and in the IC system shows the amplification in line with the specification, but the sample (see Tab. 10, example sample 2) does not show an amplification in the detection system, it means that the sample contains human DNA, but this sample is HLA-B27 negative.

If the Positive Control in the detection system and in the IC system shows the amplification in line with the specification, but the sample (see Tab. 10, example sample 3 or example sample 4) does not show an amplification of the IC, then either the DNA was not added or an unsuitable template DNA (quality, PCR inhibitor) was used. The extracted sample should be re-amplified or the isolation and cleaning of the sample should be improved.

The proof of the Positive Control and the human samples must therefore, be positive in the detection channel IC (see section 10, Quality control).

12. Limitations of the method

1. The test is not to be used for tissue typing.
2. The results which are obtained must always be interpreted in combination with the complete clinical symptoms.
3. This test is only valid for whole blood EDTA samples.
4. The listed HLA-B27 types (HLA-B*27:01 to 21, 23 to 152 and 154 to 164) were determined as 100 % detectable by using the *in silico* examination with the IPD-IMGT/HLA database (www.ebi.ac.uk/ipd/imgt/hla/) (version: October 2017). A regular comparison with the database is carried out, however it is not possible to guarantee whether additional data was added or removed from the database in the mean time.
5. The presence of PCR inhibitors cannot lead to evaluable results.
6. The German Genetic Diagnostics Act (GenDG) requires a thorough explanation and written consent of the patients for all genetic analyses in accordance with the GenDG.

13. Performance characteristics

13.1 Clinical performance characteristics

The RIDA®GENE HLA-B27 assay was validated for human whole blood EDTA samples. Please note that at the correct filling level, the EDTA concentration in a standard blood collection tubes (e.g. Sarstedt Monovette® KE/9 ml) must amount to 1.6 mg per ml of whole blood. While testing interfering substances, a concentration of 1.8 mg per ml K_2EDTA was added in addition. No interference with the following substances was detected (see Tab. 11):

Tab. 11: List of substances and concentrations used in the test

Substances	Concentrations
Heparin	15 U/ml
Cholesterol	3.0 mg/ml
Bilirubin	0.1 mg/ml
Hemoglobin	0.2 mg/ml
K_2EDTA	3.4 mg/ml

14. Version history

Version number	Chapter and designation
5/18/2018	Release version

15. Explanation of symbols

General symbols

	In-vitro diagnostics
	Observe the instructions for use
	Lot number
	Use before
	Storage temperature
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

16. Literature

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