DNA-TECHNOLOGY

561-4 2024-04-22

CE IVD

For professional use only

HLA-B27 REAL-TIME PCR Genotyping Kit

INSTRUCTION FOR USE

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

REF

R1-H004-23/4EU

R1-H004-S3/4EU

R1-H004-N3/4EU

VER

561-4.2024.04.22

TABLE OF CONTENTS

1. INTENDED USE

The **HLA-B27 REAL-TIME PCR Genotyping Kit** is intended for research and diagnostic applications. The **HLA-B27 REAL-TIME PCR Genotyping Kit** is an *in vitro* Nucleic Acid Test (NAT) – human genotyping-based product. The **HLA-B27 REAL-TIME PCR Genotyping Kit** is designed for rapid group-specific detection of HLA-B27 alleles (major histocompatibility complex, class I, B) with an aid of Polymerase Chain Reaction (PCR) method. These alleles are generally recognized as a genetic marker of multiple disease conditions e.g. rheumatoid arthritis and ankylosing spondylitis (Bekhterev's disease). Samples are human biological materials: peripheral blood.

Indications for the use:

- the presence of clinical symptoms of spondyloarthropathies: inflammatory back pain, asymmetric peripheral oligoarthritis, mainly of the lower extremities, enteritis and/or tendosynovitis;
- as an additional laboratory indicator for predicting the severity of spondyloarthropathies.

The application of the kit does not depend on population and demographic aspects. There are no other contradictions for use of the **HLA-B27 REAL-TIME PCR Genotyping Kit**.

The **HLA-B27 REAL-TIME PCR Genotyping Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin is melted or thermal dissociation of a complex of Taq-polymerase and antibodies is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **HLA-B27 REAL-TIME PCR Genotyping Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

PCR-mix includes the Internal control (IC B27). IC B27 serves as sample intake control and allows to evaluate the quantity of genomic DNA. It is needed for assurance of PCR quality and sufficiency of input DNA. The use of internal control allows to avoid false negative results in case of insufficient amount of DNA in the sample for the analysis.

DNA probe used for the detection of the HLA-B27 product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of two fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
HLA-B27	IC B27	-	-	-

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **HLA-B27 REAL-TIME PCR Genotyping Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

3. CONTENT

The **HLA-B27 REAL-TIME PCR Genotyping Kit** content is represented in Tables 2-3.

Table 2. The **HLA-B27 REAL-TIME PCR Genotyping Kit** content, package S (standard) for R1-H004-23/4EU and R1-H004-S3/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	960 µL (20 µL in each tube)	48 tubes or 6 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 ml	1 tube
Positive control	Colorless transparent liquid	75 µL	1 tube
Strip's caps*	6 8-caps		

*- for detection kit packaged in strips REF R1-H004-S3/4EU

Table 3. The **HLA-B27 REAL-TIME PCR Genotyping Kit** content, package U (universal) for R1-H004-N3/4EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless transparent liquid	960 µL	1 tube
PCR-buffer	Colorless transparent liquid	500 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	24 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 ml	1 tube
Positive control	Colorless transparent liquid	75 µL	1 tube

All components are ready to use and do not require additional preparation for operation.

The **HLA-B27 REAL-TIME PCR Genotyping Kit** is intended for single use and designed for 48 tests (no more than 46 defined samples, one positive control and one negative control)**.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

Blood sampling equipment is required:

– For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL.

Please use only salt of EDTA as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

– Biological safety cabinet class II;

– Vortex mixer;

- Refrigerator;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID Genetics** REF P-021/4EU or **PREP-GS Genetics** REF P-023/4EU are recommended);
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 65-98 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for strips;
- PCR tube rack for 0.2 mL tubes;
- 0.2 mL tubes;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of the **HLA-B27 REAL-TIME PCR Genotyping Kit**, except the TechnoTaq MAX polymerase, must be stored at temperatures from 2 °C to 8 °C during the storage period. PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period. The TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit can be transported by all types of roofed transport at temperatures from 2 °C to 8 °C over the transportation. It is allowed to transport TechnoTaq MAX polymerase at temperatures from 2 °C to 8 °C for no more than 5 days.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired the **HLA-B27 REAL-TIME PCR Genotyping Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **HLA-B27 REAL-TIME PCR Genotyping Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **HLA-B27 REAL-TIME PCR Genotyping Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be 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 dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip 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. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Amplification products must be handled in such a 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 exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **HLA-B27 REAL-TIME PCR Genotyping Kit** is designed to detect DNA extracted from the peripheral blood.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 2 – 3 times.

It is not allowed to use heparin as an anticoagulant.

Transportation and storage of the samples

Samples may be transported and stored at temperatures from 2 °C to 8 °C for no more than 24 h. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at a temperature of minus 20 °C for one month.

The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements cited in **PREP-RAPID Genetics** and **PREP-GS Genetics** extraction kits user manuals.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out according to the extraction kit instructions. **PREP-RAPID Genetics** and **PREP-GS Genetics** extraction kits are recommended. The DNA extracted with aid of **PREP-RAPID Genetics DNA Extraction Kit** should be stored no more than one month. The **PREP-GS Genetics DNA Extraction Kit** is intended for long-term storage of the extracted DNA (up to 6 months). It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control in volumes as indicated.

Assay procedure

8.1 Preparing PCR for package S

The reagents and tubes should be kept away from direct sun light.

In case of using tubes in strips, strictly observe the completeness of the strips and caps for them. Do not use the caps to the strips of the other kits!

8.1.1 Mark the required number of tubes with PCR-mix for each sample to be tested, for positive control (C+), for negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.1.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.1.4 Add one drop (~20 µL) of mineral oil into each tube. Close the tubes/strips.

8.1.5 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

In case of using **PREP-GS Genetics DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next one to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.1.6 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.1.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.8 Spin tubes/strips for 1-3 seconds.

8.1.9 Set the tubes/strips into the Real-time Thermal Cycler.

8.1.10 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (8.1.9) and run PCR. See Table 4.

Table 4. The PCR program for DTlite and DTprime Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10[1]	…	…	Holding		Holding
√ - optical measurement [1] – holding at 25°C is allowed						

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.2 Preparing PCR for package U

The reagents and tubes should be kept away from direct sun light.

8.2.1 Mark the required number of 0.2 mL tubes for each test sample, positive control (C+) and negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for "C-" and 1 tube for "C+". The resulting number of tubes is 6.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3 Add 20 µL of PCR-mix into the marked tubes.

8.2.4 Vortex the TechnoTaq MAX polymerase and PCR-buffer for 3-5 seconds, then spin for 1-3 seconds.

TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:

- 10 x (N+1) µL of PCR-buffer,
- 0.5 x (N+1) µL of TechnoTaq MAX polymerase,

N is a quantity of the samples to be tested taking to account "C-", "C+".

Example: for simultaneous testing of 5 samples, "C-" and "C+" in one PCR run, mark 7 tubes (5 tubes for samples to be tested, 1 tube for "C+" and 1 tube for "C-"). Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase for 8 (7+1) tubes. Mix 80 µL of PCR-buffer and 4.0 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase (3-5 seconds) and spin briefly in vortex mixer (1-3 seconds).

Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 10 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube with PCR-mix.

Follow the steps listed in pp. 8.2.8 – 8.2.14 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Add one drop (~20 µL) of mineral oil into each tube. Close the tubes.

8.2.9 Vortex the tubes with samples, "C+" and "C-" for 3-5 seconds and spin down drops for 1-3 seconds.

In case of using **PREP-GS Genetics DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. Close the tubes tightly. Use filter tips.

8.2.10 Add 5.0 µL of DNA sample into corresponding tubes. Do not add DNA into the "C+", "C-" tubes. Avoid paraffin layer break.

8.2.11 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube.

8.2.12 Spin tubes for 1-3 seconds.

8.2.13 Set the tubes into the Real-time Thermal Cycler.

8.2.14 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes in the thermal unit (8.2.13) and run PCR. See Table 4.

9. CONTROLS

The **HLA-B27 REAL-TIME PCR Genotyping Kit** contains positive control sample. Positive control is a cloned part of the gene detected by the aid of the kit. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. PCR-mix includes the internal control (IC B27). ICB 27 serves as sample intake control and allows to evaluate the quantity of genomic DNA. It is needed for assurance of PCR quality and sufficiency of input DNA.

To reveal possible contamination a negative control is required.

A negative control sample should go through all stages of DNA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

The test result is considered valid when the software represents a graph with the fluorescence dependence of the cycle number for each tube in the thermoblock. The table will show the sample ID and the Cp for Fam and Hex.

The test result is considered invalid when the Cp of Hex is more than 32.0 or absent.

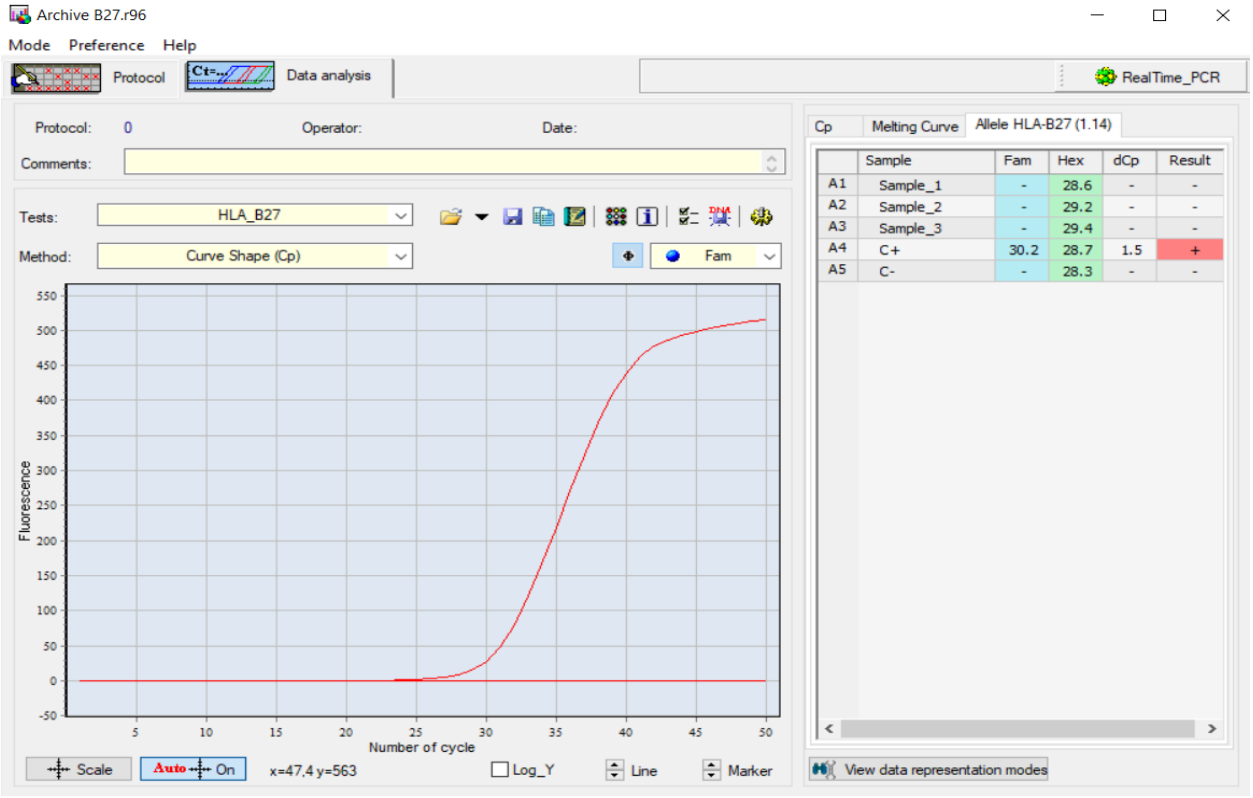
If positive control (C+) does not have positive result, it is necessary to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling requirements.

If negative control (C-) has positive result, all results of current PCR run are considered false. Decontamination is required.

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

10. DATA ANALYSIS

Registration and interpretation of the PCR results are held in automatic mode. The graph will show the fluorescence dependence of the cycle number for each tube in the thermoblock. The table will show the identification of the sample and the Cp for Fam and Hex.



	Sample	Fam	Hex	dCp	Result
A1	Sample_1	-	28.6	-	-
A2	Sample_2	-	29.2	-	-
A3	Sample_3	-	29.4	-	-
A4	C+	30.2	28.7	1.5	+
A5	C-	-	28.3	-	-

The interpretation of the result for each sample is carried out automatically with respect to Cp for Fam (specific dye label) and Hex (internal control dye label) channels.

HLA*B27 gene detection

DNA-TECHNOLOGY

Tube number:
Patient:
Sex:
Age:
Physician:
Comment:

Information about laboratory

Sample ID: C+

Name of research	Result
allele HLA-B27	Detected

Study was carried out by

Date
Signature

Table 5. Cp for Fam and Hex channels used for interpretation of PCR results (only for DTlite or DTprime instruments)

Fam (Fam Cp)	**Hex (Hex Cp)**	**ΔCp = Cp (Fam) - Cp (Hex)**	**Interpretation**
Cp is defined	Cp ≤32*	Less than 8.0	HLA-B27 is detected
Cp is defined	Cp ≤32	More than 10.0	HLA-B27 is not detected
Cp is not defined	Cp ≤32	Is not considered	
Cp is defined	Cp ≤32	8.0 – 10.0	Doubtful
Cp is defined/not defined	Cp is not defined	Is not considered	Unreliable
	Cp > 32	Is not considered	
* corresponds to the 1.0 ng of genomic DNA per amplification tube.			

In case of obtaining unreliable results, the procedure starting from the PCR amplification step must be repeated. If new test will confirm the unreliable result, the DNA extraction and PCR amplification or/and sampling procedure must be repeated (is performed sequentially).

Unreliable result can be related to the presence of PCR inhibitors in DNA sample, incorrect performance of the analysis protocol, violation of the amplification temperature regimen, etc.

If DNA sample was obtained with **PREP-RAPID Genetics DNA Extraction Kit** and ΔCp = Cp (Fam) - Cp (Hex) value falls within 8.0-10.0 range the DNA sample should be diluted 10 fold by distilled water. Take into account that Cp value by Hex channel will change. In this case the result should be considered reliable when Cp <35.0.

The positive control sample must contain the HLA-B27 allele. If the positive control sample has negative or unreliable result, the results of the whole PCR run are considered to be unreliable. In this case, the PCR amplification step for samples in current PCR run is required to be repeated.

The negative control sample must have an unreliable result. Receiving different values, the results of whole PCR run are considered to be unreliable. In this case, it is necessary to carry out special measures to eliminate contamination.

11. SPECIFICATIONS

a. The analytical **specificity** of the **HLA-B27 REAL-TIME PCR Genotyping Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

If samples contains HLA-B27 allele in homozygous or heterozygous state, a positive result is recorded via Fam and Hex channels, provided that Cp Hex ≤ 32 and ΔCp = Cp (Fam) – Cp (Hex) is less than 8.0.

If samples do not contain allele HLA-B27, a negative result is recorded via FAM channel and a positive result is recorded via Hex channel (Cp Hex ≤ 32), or a positive result is recorded via Fam and Hex channels, provided that Cp (Hex) ≤ 32 and ΔCp = Cp (Fam) – Cp (Hex) is more than 10.

b. In a determination of analytical **sensitivity,** the **HLA-B27 REAL-TIME PCR Genotyping Kit** demonstrated the ability to reproducibly detect 1 or more genome equivalents per PCR reaction.

The lower limit of detection is not less than 1.0 ng of human DNA per amplification tube, which corresponds to Cp≤32 on the IC detection channel. When the amount of DNA is smaller (Cp>32 on the IC detection channel), the manufacturer does not guarantee the correct result of the kit.

After the amplification reaction for samples with insufficient quantity of DNA (less than 1.0 ng per amplification tube), the result is defined as unreliable.

c. Diagnostic characteristics

Number of samples	**N=150**
Analytical sensitivity (95% CI)	100% (94.1-100%)
Analytical specificity (95% CI)	100% (95.9-100%)
Diagnostic sensitivity (95% CI)	100% (92.9-100%)
Diagnostic specificity (95% CI)	89% (81.2-94.4%)

The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID Genetics** REF P-021/4EU or **PREP-GS Genetics** REF P-023/4EU kits.

12. TROUBLESHOOTING

Table 6. Troubleshooting

	Result	**Possible cause**	**Solution**
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issueswith the kit:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **HLA-B27 REAL-TIME PCR Genotyping Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

https://www.obelis.net

14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version		Do not reuse
NON STERILE	Non-sterile	CONTROL +	Positive control
EC REP	Authorized representative in the European Community		Caution

REF

R1-H004-23/4EU

R1-H004-S3/4EU

R1-H004-N3/4EU

VER

561-4.2024.04.22