HLA B-27 Molecular Diagnostic Kit

Molecular Genetic DNA Typing SSP Technique

For In Vitro Diagnostic Use

By ROJE
Edition, 01/2022

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

Contents

Kit Content

Components	48 reactions	96 reactions
B-27 Master Mix, 2X	123µl	247µl
B-27 Primer Mix	312µl	624µl

Introduction

Ankylosing spondylitis (AS) is a type of arthritis and autoimmune in which there is a long-term inflammation of the joints of the spine. Typically the joints where the spine joins the pelvis are also affected. Occasionally other joints such as the shoulders or hips are involved. Eye and bowel problems may also occur. Back pain is a characteristic symptom of AS, and it often comes and goes and men infected more than women. Stiffness of the affected joints generally worsens over time.

Storage

Shipment condition is checked by ROJETechnologies. After arrival, all components should be kept dry at -20°C. When storage condition is as directed, all components are stable until expiration date, as indicated on the kit box.

Intended Use

HLA B-27 Molecular Diagnostic Kit is an in vitro nucleic acid amplification test for rapid group-specific detection of HLA B-27 alleles of human major histocompatibility complex in the biological materials (whole blood and oropharyngeal swabs). Notice that, assay for the molecular typing of HLA B-27 is based on polymerase chain reaction (PCR). We recommend all users to study PCR experiments guideline, before starting their work.

Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our

technical support team. If problem is due to manufacturing process, ROJE team will replace the Kit for you.

Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell it or any components. For information about out licensing or distributors contact ROJE business team.

Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at www.rojetechnologies.com.

Quality Control

All ROJETechnologies products are tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at www.rojetechnoloes.com.

Description

HLA B-27 Molecular Diagnostic Kit is designed to determine HLA B-27 alleles based on the Polymerase Chain Reaction method using sequence specific primer (PCR-SSP assay), which enables amplification of defined DNA sequences. The principle of the SSP assay is to generate an amplicon only when the sequence of a primer is perfectly complementary to the target sequence of a DNA sample. On the other hand, non-complementary primer do not bind to the DNA and then no amplification takes place. Evaluation of the result is performed by agarose gel electrophoresis. In the electric field the amplicons separate according to their size. If no amplification takes place, no specific band is seen. This kit is tested for 1094 individuals and has shown %100 concordance compared to sequencing, showing %100 specificity and sensitivity in this population size.

Procedure

Primer pairs are designed for typing HLA B-27 alleles. HLA locus-specific amplification is performed in a thermal cycler using the amplification primer mix, template DNA, and the B-27 Master Mix, 2X. After successful amplification, the genomic DNA target sequence is present in a detectable concentration. In all reaction an internal control primer pair exist, which amplifies a conserved region of a housekeeping gene in every PCR reaction mix showing if the PCR reaction had taken place and to exclude results based on inhibitors, non-sufficient DNA and displaced primers from other wells. After the PCR process, the amplified DNA fragments are size-separated by agarose gel electrophoresis and exposure to ultraviolet light, documented by photography and interpreted. Interpretation of PCR-SSP results is based on the presence or absence of specific PCR product(s). The relative sizes of the specific PCR product(s) may be helpful in the interpretation of the results.

Equipment & Reagents to Be Supplied by User

- Thermal Cycler
- Pipets and pipet tips
- Microwave oven / Heating Equipment
- Agarose gel electrophoresis system
- PCR tray rack
- Mineral oil (for thermal cyclers without a heated lid)

Instrument Requirements

Thermal Cycler

Thermal cyclers which have no adjustable pressure plate require an adaptor mat in order to guarantee optimal heat transfer from the heat cover to the PCR tubes. For optimal results it is important to obtain rapid ramp times (1°C-2.5°C/s) and precise temperature control. The PCR program is shown in the Table 3.

Gel Electrophoresis

The accomplishment of the gel electrophoresis is described in "Agarose gel electrophoresis".

Application

- Population studies
- Disease associations
- Pharmacogenomics

Features

Specific features of HLA B-27 Molecular Diagnostic Kit are listed here in Table 1.

Table 1: HLA B-27 PCR Kit features and specifications

Features	Specifications
Enzyme	Recombinant Taq Polymerase
Reaction type	PCR Amplification
Sample	gDNA, cDNA
Single / Multiplex	Multiplex

Sample storage and Preparation

DNA Sample Requirement

Extracted, highly pure DNA is needed for SSP typing:

- DNA samples to be used for PCR-SSP HLA typing should be re-suspended. To prepare a working solution of the purified DNA at 15-200ng/µl, dilute the DNA in TE Buffer or RRB Buffer (BU983005). DNA can also be diluted in water for immediate use. DNA aliquoted in water should be discarded after use. TE or RRB (BU983005) Buffer is recommended for long term storage.
- DNA can be extracted using all of the traditional extraction methods. Please ensure that the OD260/280 of the DNA sample falls between 1.66 and 1.94 as measured by UV spectrophotometry.
- We recommend DNA extraction with the DNJia Blood and Cell Kit Cat No DN983025 or DNSol Kit Cat No DN983002, ACD blood should be used as starting material. Alternatively, the DNA can be extracted by any preferred method yielding pure DNA. When using alternative methods, the DNA concentration should be adjusted to 15-200 ng/µl. Do not use heparinized blood with these methods.

- Each reaction in the test is optimized to utilize between 100 – 500 ng of DNA.
- Concentrations lower than 50ng/µl will increase the risk for weak extra bands.
- DNA samples should not be re-suspended in solutions containing chelating agents such as EDTA, above 0.5mM in concentration. (Ensure the final DNA sample does not contain more than 2.5mM Tris/0.25mM EDTA. Only use DNA extracted from citrate and EDTA collected samples. As heparin may inhibit PCR it is recommended that DNA should not be extracted from heparinized blood samples.)
- DNA samples may be used immediately after extraction or stored at +4 ºC for up to 2 weeks with no adverse effects on results. DNA samples can be stored at -20 ºC or colder for 9 months. The purity and concentration of extracted DNA samples that have been stored for a prolonged period should be tested for acceptability prior to testing.
- Avoid the use of lipemic or hemolyzed specimens. The use of specimens collected without anticoagulant or frozen/thawed multiple times is not recommended since these conditions may not provide sufficient quantity or quality of DNA for testing.
- DNA samples should be shipped at +4ºC or colder to preserve their integrity during transport.

PCR- Polymerase Chain Reaction

PCR is an extremely sensitive method which can efficiently amplify even the smallest amounts of DNA Even traces of contaminating DNA in a sample can be amplified in the PCR reaction and falsifies the test results. One particular source of contamination is amplified DNA coming into contact with samples which are still to be amplified. To avoid contamination with amplified material, it is recommended that the work areas be strictly separated as follows:

- Spatial separation of the pre-PCR area (DNA isolation, storage, PCR sample) from the post-PCR area (thermocycler, gel electrophoresis, analysis).
- Components of the post-PCR area must not get into the pre-PCR area.
- When working in the pre-PCR area, pipettes with aerosol protection should be used (filtered tips).
- It is recommended that a negative control be included in the test procedure as an indication of contamination with foreign DNA.

Before Start

- The test must be performed by well-trained and authorized laboratory technicians.
- Do not use reagents past the expiration date printed on the label.
- Do not use reagents with any evidence of turbidity or microbial contamination.
- The Control DNA is extracted from a standardized reference human B-lymphoblastoid cell line. The extracted DNA uses a number of enzymatic steps to minimize exposure to potentially infectious material.
- Pre- and post-PCR activities should be separated according to good PCR laboratory procedures.
- Do not use primer trays with cracks in the wells or damage to the upper rim of the wells as this may cause evaporation during PCR amplification. Do not use PCR cap strips with cracks as this may cause evaporation during PCR amplification.
- DNA samples should be shipped at +4ºC or colder to preserve their integrity during transport.

Buffer Preparation

- Add 2.56µl B-27 Master Mix to the tube.
- Add 6.5µl of B-27 Primer Mix.
- Add 100-500ng of your sample to each well, then pipette up and down (Table 2).
  Note: Do not use lower DNA concentrations.
- Spin down the solution and close the tube strips lids or seal the plate properly with the PCR adhesive foil.
- Put the Tubes/plate into the thermal cycler and start the program (see Table 3).

Table 2. Prepare PCR Reactions for HLA B-27

Component	Volume (1 Reaction)
B-27 Master-Mix	2.56µl
B-27 Primer Mix	6.5µl
Sample	100-500ng

ddH2O	To 12µl
Final Volume	12µl

Preparation of the PCR for HLA B-27

Table 3. PCR Program for HLA B-27

Cycles	Step Name	Temperature	Duration
--	**Initial Incubation**	25°C	3 min
--	**Initial Denaturation**	96°C	2 min
10 Cycles	**Denaturation**	94°C	10 sec
	Annealing	65°C	1 min
20 Cycles	**Denaturation**	94°C	10 sec
	Annealing	61°C	50 sec
	Extension	72°C	30 sec
--	**Final Extension**	72°C	5 min
--	**Store**	4°C	----

Agarose Gel Electrophoresis

The PCR products are identified using agarose gel electrophoresis followed by detection of the DNA bands in UV light.

Preparing 2% (W/V) Agarose Gel

- Dissolve 2.0 grams of electrophoresis grade agarose powder in 100 ml of 1X TAE buffer (TAE, 10X, Filtered, Sterile, Molecular Cat NO: BU983104).
- Melt the agarose powder completely by boiling in a microwave oven or on a heating apparatus. Stir constantly. If evaporation occurs, replenish with ddH2O.

Casting Gel

- Cool the heated agarose gel to ~60 ºC.
- Add at least 2µl of safe stain to the heated agarose. Stir until it is thoroughly incorporated.
- On a balanced surface, set up a gel plate with combs.

- Cast a 5mm thick gel on the plate.
- Allow the gel to settle.

Gel Electrophoresis

- Submerge the gel in 1X TAE buffer in a gel box.
- Gently remove the strip lids without splashing the PCR products.
- Load a 5 to 8 µl of PCR product into each well on the gel.

  Note: The PCR products of HLAB-27 are stained with cresol red. There is no need to load additional "loading buffer" or dye.

  Optional: Reserve the first well on each row for molecular weight standards, in increments of 100bp from 50 to 1000bp.
- Connect the electric leads and turn on the power supply (115V AC). Electrophoresis for ~ twenty (25) minutes, or until two thirds (2/3) of the lane.
- Turn off the power supply, and remove the gel from the gel box.
- Transfer the gel tray with the gel to a UV transilluminator.
- Photograph the gel with or without the gel tray.
- Mark the photograph according the rules of the laboratory.

Results

Evaluation

PCR products specific for HLA and belonging to the internal control become visible in the gel under UV light. The HLA B-27 primer mixture contains control primers which amplify a 434 bp fragment of human growth hormone (HGH). The concentration of these primer pairs is lower than the allele specific primer pairs and their purpose is to provide an internal control of successful PCR amplification. This amplification generally always occurs, i.e. both in presence or in absence of an allele-or group-specific PCR fragment. The control band can therefore generally be seen in all PCR reactions. From time to time, the control band can appear weak or is completely missing in the presence of an allele-specific HLA PCR product. This is not a limitation of the method, as the specific band provides a check on the success of the PCR amplification. The composition of the primers permits positive identification of the HLA characteristics. The interpretation is based on whether a specific band is present on the gel or not. The size of the amplified DNA fragments does not need to be taken into consideration when evaluating the test, nevertheless it might be

helpful for the test interpretation. For evaluation, the pattern of the specific bands is transferred to the result sheet supplied and the typing result read off with the aid of the reaction pattern.

Gel Interpretation

Reaction	Positive	Positive	Positive	Negative	No amplification	Size (bp)
Gel lane						
Control band					None	434
Specific band		None		None	None	234
Specific band			None	None	None	142
Primer dimers						≤100

Figure 1: schematic diagram of possible results of B-27 typing at the gel analysis

For the interpretation of the results it is important if a band is present in the gel or not. The composition of those positive bands identifies the HLA characteristics. The size of the fragments (using a molecular weight standard) can help to interpret the results, but it is not essential for the evaluation of the test. This way it is ensured that the results are not based upon false positive bands caused by unspecific reactions or specific primers carried from one well to another.

Test Limitation

- Because of the high sensibility of the PCR the purity of the reagents should be obtained by working accurately.
- Performance of the test can only be guaranteed if enclosed instructions are strictly adhered to.
- The use of other Master mixes or PCR sets can lead to failures as well as to non-specific false positive PCR amplifications.
- DNA samples may be used immediately after isolation or stored at –20°C or below for extended periods of time (over 1 year) with no adverse effects on test results.

symbols

symbols	meaning	symbols	meaning
	Hazardous chemicals		manufacturer
	Date of manufacture		Temperature limitation
	Use by	LOT	Lot number
REF	Reference number	IVD	In Vitro Diagnostics

Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE Technical Support Team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
DNA "smear "in the gel tracks; Background bands; Non-specific PCR product	DNA concentration problem	• Check the DNA concentration is neither too high nor low. Aim for between 50 - 100ng of DNA per reaction.
	Impure or degraded DNA	• Measure OD260/OD280 (a quotient of 1.8 is optimal) and evaluate on agarose gel. Re-extract DNA if necessary.
	Contamination	• Check negative control. Check sample DNA and re-extract if necessary. Use filter tips.
	Incorrect amplification conditions	• Check the thermocycler.
	Primer dimer bands evaluated as positive specific reactions.	• Check band sizes.
Weak bands; Suppression of reactions	Use of insufficient amount of DNA.	• Double the amount of DNA (reduce dH2O in preparation); use around 100 ng DNA per PCR preparation
	Impure or degraded DNA	• Measure OD260/OD280 (a quotient of 1.8 is optimal) and evaluate on agarose gel. Re-extract DNA if necessary.
	Master mix or Sample DNA not added to PCR Mix	• Repeat PCR.
	Insufficient mixing of the DNA or reactions	• Dissolve DNA at 37-65°C. Vortex well after preparing the master mix.
	Tubes not correctly sealed	• Check the seal of the cover strips or PCR mat (use press-on mat).

	Incorrect amplification conditions	• Check the thermocycler.
	PCR inhibitors such as ethanol, hemoglobin, heparin, beads contained in preparation.	• Use EDTA or citrate blood as base material; (after the DNA pellet has been washed with ethanol, ensure that it is sufficiently dry).
	pH value of the DNA solution is too acidic (PCR cocktail changes color after adding DNA)	• Precipitate DNA once more and dissolve in nuclease free water.
	PCR product leaked out of gel pocket	• Correct gel preparation with straight pipette tips.
Bands in the PCR mix of the negative control	By mistake DNA was pipeted into the mix of the negative control	• Possibly repeat preparation or make a note of it in the evaluation documents
	Contamination of the reagents	• Exchange reagents
Amplification pattern is not interpretable	Incorrect interpretation of an artefact as a specific band	• Check the specific Interpretation Tables for correct band size. • Check if all specific amplifications are correct in size or if an artefact (carry-over, primer dimer) has been misinterpreted as an amplification
	Reactions loaded in the incorrect order	• Check alignment of PCR and gel lanes.
	Individual PCR failure	• Check all internal positive controls are present. Reinterpret without any missing reactions.
	Small amplicons missing	• Electrophoresed too far, small amplicons have run off the end of the gel, or past the ethidium bromide front, or are dispersed by entering preceding gel well. Use electrophoresis conditions suitable for your gel system.
	New allele identified in sample	• New alleles may occasionally be discovered that may give rise to an amplification pattern that does not correspond to an existing allele(s). Please contact ROJE Technical Support Team.

Ordering Information

Category	Product Name	Cat NO.	Size
Molecular Diagnostics	HLA B-27 Molecular Diagnostic Kit, SSP-PCR assay	MD983046	48 Reactions
	HLA B-27 Molecular Diagnostic Kit, SSP-PCR assay	MD983045	96 Reactions
	HLA B5 and B51 Molecular Diagnostic Kit, SSP-PCR assay	MD983051	48 Reactions
	HLA B5 and B51 Molecular Diagnostic Kit, SSP-PCR assay	MD983052	96 Reactions
	HLA B5 Molecular Diagnostic Kit, SSP-PCR assay	MD983047	48 Reactions
	HLA B5 Molecular Diagnostic Kit, SSP-PCR assay	MD983048	96 Reactions
	HLA B51 Molecular Diagnostic Kit, SSP-PCR assay	MD983050	48 Reactions
	HLA B51 Molecular Diagnostic Kit, SSP-PCR assay	MD983049	96 Reactions
	DNSol Mini Kit	DN983003	100 Preps
	DNSol Midi Kit	DN983014	50 Preps
	DNSol Maxi Kit	DN983018	50 Preps
	DNSol Clotted Blood Kit	DN983032	50Preps

Technical Assistance

ROJETechnologies guarantees your complete satisfaction. ROJE technical support team composed of highly trained experienced scientists, who are able to troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select suitable product.

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number; +982191070705.

- You can submit your question directly to ROJE technical support team from our website (www.rojetechnologies.com).
- Or send your questions to this email address, technicalsupport@rojetechnologies.com.

Appendix 1: Preparation of 50x TAE Buffer

Dissolve the components in about 1000ml dH2O. Adjust the pH to 8.3 with NaOH. Adjust the volume to one liter. Store at room temperature. You can also order TAE, 50X, Filtered, Sterile, Molecular Biology Grade by Cat. No. BU983028. 1:49 final dilution of the 50x TAE buffer solution in ddH2O or you can order Water for Molecular Biology, Sterile, Filtered by Cat. No. WA983009. The solution is used for running buffer and the preparation of the gel.

Table 4. Preparation of 50x TAE buffer

Component	g/Litter
Trizma® base	242g
Glacial acetic acid	57.1ml
0.5 M EDTA (pH 8.0)	100ml

Factory address:

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130- phone: +982191070705