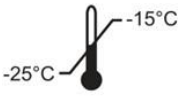
Instructions For Use – version 2

For In Vitro Diagnostic Use



Store at -25ºC to -15ºC

B51 - RT50

50

geneMAP™ HLA-B*51 Detection Kit

For Real-Time PCR

The Realtime PCR Kit can detect HLA-B*51 Allele in Human Genomic DNA.

,

Validated on:

* Biorad® CFX96, Real-time PCR System (Bio-Rad)
* Life Technologies ABI Prism® - 7500, Step-One & QuantStudio Series
* Qiagen Rotor-Gene® 3000 Q5/Q6
* Roche, LightCycler® 480 II, Cobas Z480
* BioMolecular Systems, MicPCR

Contents

1. Intended Use

HLA-B*51, class I human leukocyte antigen (HLA) molecule is the strongest genetic risk factor known for Behçet's Disease (BD). The kit is a test for detecting the presence of the most frequent type HLA-B*51 allele from the human genomic DNA. (1)

2. Safety Instructions & General Warnings

- This kit must be used strictly in accordance with the instructions provided in this manual, and only in combination with validated reagents and instruments. Any off-label use of this product, as well as any modification of its components, will nullify Genmark's liability.
- Obey proper laboratory safety protocols when working with chemicals and specimens.
- The protocol can be performed by only professional and trained personal.
- Perform the protocol in a well-ventilated and well-lit environment.
- Store the kit and its components within recommended temperature range in de-frost refrigerators. Do not use no-frost refrigerators.
- Avoid skin contact with any of the reagents in the kit
- Wipe workspace surfaces with 10% bleach followed by 70% alcohol.
- Instruments may exhibit performance variations due to differences in electrical currents and power outlets, as well as the effects of maintenance and calibration. It is the responsibility of the user to ensure that all instruments are properly maintained and regularly calibrated according to the manufacturer's guidelines. Genmark disclaims responsibility for any performance issues arising from improper maintenance, calibration, or variations in electrical supply.
- All biological specimens should be handled as potentially infectious, following standard precautions. For guidelines on specimen handling, refer to the World Health Organization.
- Consult environmental waste personnel for proper disposal of used plates, consumables, and reagents, considering federal and local hazardous waste regulations. Check local and national disposal requirements.

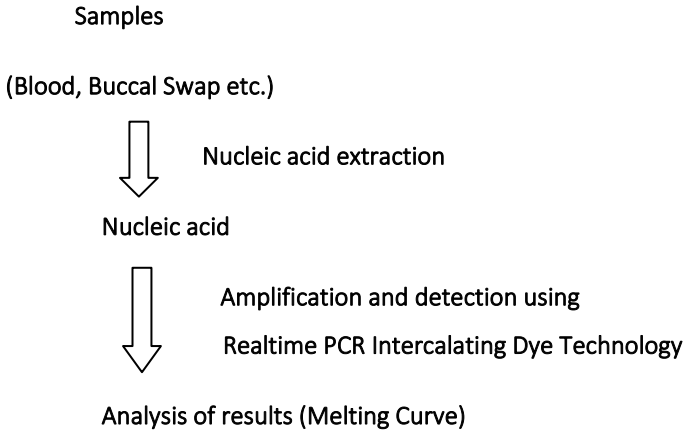
3. Principles and Procedure Overview

3.1 Principles

The Real-time polymerase chain reaction (Real-time PCR) is sensitive and specific technology based on DNA amplification technique. The kit is based on two main processes: nucleic acid extraction and Real-time PCR melting curve analysis.

The kit targeted for amplification of HLA-B*51 allele and Endogenous Control (EC) / Beta Globin of Human DNA.

Procedure Overview;



3.2 Technology

In the presence of target alleles, the realtime amplification curves are generated by allele specific PCR primers then mutation genotyping is done by melting curve analysis.

Melting curve analysis is an assessment of the dissociation-characteristics of double-stranded DNA during heating. As the temperature is raised, the double strand begins to dissociate leading to a rise in the absorbance intensity, hyperchromicity. The temperature at which 50% of DNA is denatured is known as the melting point, though it is an inaccurate term as it has very little to do with a traditional melting point.

4. Background Information

Each isolated DNA should be tested with multiplex (Endogenous control and HLA-B*51 specific primers) Real-time-PCR primer mixes. The test system is designed for use with sequence specific primers. The fluorescence dye of mutation analysis is EvaGreen (Tm- Biotium).

5. Reagents

Reagents contained in a kit are sufficient for **50** reactions.

Description	No. of Reactions*	No. of Tubes	Vol. Per Tube	Color of Caps	Contents
10X HLA-B*51 Primer Mix	55	1	110 µl	Green	• Amplification and detection reagents • Template of Endogenous Control
2X Master Mix (with Evagreen)	55	1	550 µl	Amber	• DNA polymerase with UDG • Buffer containing dNTPs • Green Intercalating Dye
RNase Free Water	80	1	400 µL	Violet	• RNase Free Water
HLA-B*51 Positive Control	16	1	50 µL	Red	• Synthetic DNA/Ultramer

***Note:** Do not subject the tubes to more than 5 freeze-thaw cycles.

6. Storage and Handling

All components of geneMAPTM HLA-B*51 Detection Kit Kit must be stored at between -15° C /-25 ° C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components is not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

7. Materials Required But Not Provided

- Disposable powder-free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipettetips
- 1.5mL microcentrifuge tubes
- Heat block
- Desktop centrifuge
- Vortex mixer
- Clean bench

8. Protocol

8.1 Specimen Collection, Storage and Transport

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation.

8.2 Nucleic Acid Extraction

Various manufacturers offer nucleic acid extraction kits. Use the correct protocol according to the manufacturers' protocol. You can use any commercial DNA extraction kit or conventional DNA extraction method (Phenol-Chloroform) from Blood or Buccal swab. The optimal concentration of DNA should be between 20ng/µl and 200 ng/uL.

8.3 Preparation for Real-time PCR

* The correct tubes and caps must be used.
* Aerosol resistant filter tips and tight gloves must be used when preparing one-step RT-PCR reactions. Use extreme care to ensure no cross-contamination.
* Briefly centrifuge the reagent tubes to remove drops from the inside of the cap.

Thaw all of the primer mix, positive control mix, RNase Free Water and 2X reaction master mix. Spin down the tubes at room temperature.

The PCR reactions are setup in a total volume of 20 µl/sample. Reaction mixes for multiple samples (as well as control samples) should be pre-mixed as a master mix with 2% overage to cover pipetting errors.

The following reagents go into each 17 µl reaction;

Reagents	1X Rxn Volume (µl)
2X HLA-B*51 Master Mix	10 µl
10X Primer Mix	2 µl
RNase Free Water	5 µl

* Dispense 17µl of the master mix per well into a single well.
* Add 3 µl of each sample DNA or PC or RNase Free Water as NTC to its corresponding well.
* ABI Prism® system, please choose "none" as passive reference and quencher.
* Seal the plate with the recommended adhesive film, or ensure tubes are well capped.
* Briefly tap the wells to ensure adequate mixing.
* Centrifuge the plate or PCR tubes to collect the contents.
* Transfer the sealed PCR plate to a proper Real-time PCR instrument and run the cycling protocol listed in following table.

9. Real-time PCR Instrument Setup and Results Analysis

9.1 Real-time PCR System

9.1.1 Pre-settings for Data Analysis

Pre-settings for Fluorophore Selection and Thermal Cycling Conditions

Targets	Reporter Fluorophore
HLA-B*51 Allele & Endogenous Control	FAM/EvaGreen/SyberGreen

Step	No of Cycles	Temperature	Duration	Ramp Rate for ROCHE LC480
1	1	95°C	15 min	4.4
2	35	95°C 60°C	15 sec 45 sec	3.5 2.2
3	1	95°C	30 sec	4.4
4	1	50°C	30 sec	4.4
5	1	70°C to 95°C	Incremenet 0,3°C for 0,05°C+plate read	Bio-Rad CFX96
	1	70°C to 95°C	for 0,05°C/sec 12 acquisitions per °C	Roche LC480
	1	70°C to 95°C	Ramp percentage 0,2%	ABI 7500, StepOne, StepOne Plus

Note1: Please use only 72-well carousel for Qiagen Rotor-Gene® Q5/Q6, 36-well carousel is not recommended. Additionally, perform Auto-Gain optimization before first acquisition. (Auto-Gain optimization tube should be PC)

10. Results

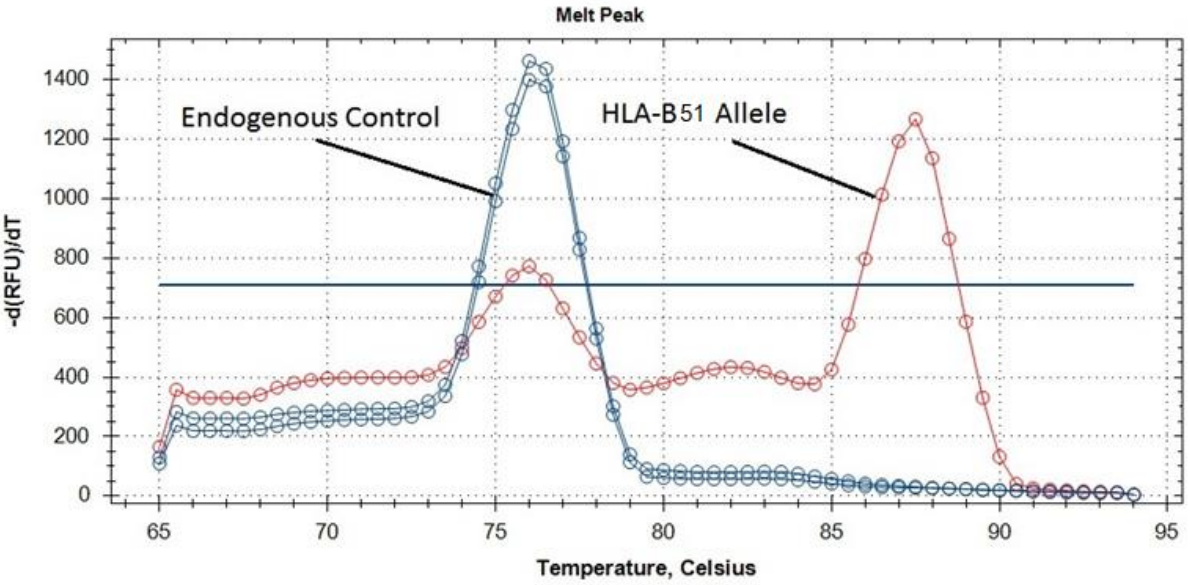
10.1 Interpretation of Results

After the run is completed data are analysed using the standard melting curve software with FAM/SyberGreen/EvaGreen dyes. The below results were studied with CFX96-BioRad.

Expected Tm of the Peaks

- Endogenous Control Peak Melting Temperature Tm: 76°C (+/- 2°C)
- HLA-B*51 Positive Melting Peak Temperature Tm: 87,5°C (+/- 2°C)

Example Melting Peaks of Normal and HLA-B*51 Positive Allele Patients



- *HLA-B*51 Negative Samples (in blue):* *Only Endogenous control peaks occurs.*
- *HLA-B*51 Positive Sample (in red):* *Both Endogenous and HLA-B*51 peaks occurs.*

11. Limitations

HLA-B * 51 contains about 200 lower allele (HLA-B * 51: 01-: HLA-B * 51: 189). The most common allele is HLA-B*51: 01. The HLA-B*51 is 63-Asn and 67-Phe motifs, which are responsible for Behçet's disease, regardless of the subletera. Although the Kit in silico has detected many subspecies, **HLA-B * 51: 05 does not detect the by this kit. This subtype was not found in the Asian and European geographic areas.**

12. References

1. Imperiali, et al. Rapid detection of HLA-B*51 by real-time polymerase chain reaction and high-resolution melting analysis, Tissue Antigenes, 2015 Aug. 86(2):139-42

13. Revision History

Date of Last Edit: December 2024		
Change	**Affected Section**	**Page**

14. Troubleshooting

geneMAP™ HLA-B*51 Detection Kit		
OBSERVATION	POSSIBLE CAUSES	SOLUTION
No signal in fluorophore	Fluorophores incompatible with protocol for data analysis	Select the correct fluorophores.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Repeat the test with the new extracted nucleic acid.
Melting curve signals in Negative Control	Cross Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips and throughout the procedure and change tips between tubes. Repeat entire procedure from nucleic acid extraction with the new set of reagents.
No melting curve signal in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre-composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please carefully repeat the test.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 4) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.

15. Symbols Used

REF	Catalog Number
LOT	Lot/Batch Number
	Expiration Date
-15°C -25°C	Storage Conditions
	Manufactured by
CE IVD	Intended Use

16. Contact Information

Genmark Sağlık Ürünleri

İthalat İhracat ve Ticaret Limited Şirketi

Halil Rıfat Paşa Mah. Güler Sok. GNM Plaza No:51-1 34384 Okmeydanı / Şişli- İstanbul

Tel: +90212 288 74 92/93

Fax: +90212 288 74 53

Email: info@genmark.com.tr ; b.eratak@genmark.com.tr Web: www.genmark.com.tr