SNP Biotechnology R&D Ltd.
Hacettepe Technopolis 1. R&D Building Office No: 10 Beytepe – ANKARA / TURKEY
Tel : +90312-2992328 / Fax : +90312-2992348 / www.snp.com.tr / info@snp.com.tr

HLA-B52 REAL-TIME PCR KIT
Cat. No: 504R-10-01

INTRODUCTION

Behçet's disease is a chronic, inflammatory, multisystem disorder predominantly affecting populations of Asian, Middle Eastern and Mediterranean. With the exception of oral aphthosis, BD is characterized by considerable phenotypic variation, comprising a myriad of manifestations, e.g. recurrent genital ulcers and skin, joint, eye, vascular and/or CNS involvement. Over the last 30 years, a substantial body of knowledge has accumulated supporting a strong genetic underpinning in BD of the MHC-related allele HLA-B5, which was later more specifically linked to its predominant suballele HLA-B52. [1,2].

INTENDED USE

The HLA-B52 Real Time PCR Kit detect all subtypes of HLA B52 in the **IMGT / HLA Gene FASTA 3.32.0 database** with high specificity[3].

TARGETED USER

For professional use only. Testing should be performed by professionals trained in molecular techniques.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5′ end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (C_T) is proportional to the amount of the specific PCR product [4,5].

PRODUCT SPECIFICATION

Each isolated DNA should be tested with mix 1 and mix 2. The kit provides reagents in a **"ready-to-use"** master mix format which has been specifically adapted to 5′ nuclease PCR for SNP analysis. The test system is designed by SNP Biotechnology for use with sequence specific primers and probes.
The fluorescence of HLA-B52 analysis is FAM. Also each master mix contains an internal control labelled with HEX/JOE dye. Internal Control is Prothrombin gene – FII (OMIM: 176930).
The limit of detection (LOD) for the HLA-B52 Real-Time PCR Kit was determined as 1 ng/µl.

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
HLA-B52 Mix 1	200 µl	400 µl	1000 µl
HLA-B52 Mix 2	200 µl	400 µl	1000 µl
HLA-B52 Positive Control DNA *	30 µl	30 µl	60 µl
HLA-B52 Negative Control DNA *	30 µl	30 µl	60 µl

Table 1: Kit content

* Since to Control DNA is a synthetic plasmid, amplification plots of synthetic control DNA may appear slightly different from the sample DNA. Please gently vortex and then spin centrifuge for 1-2 seconds before use the positive control.

SAMPLE COLLECTION

HLA-B52 Real-Time PCR Kit is approved for use with whole blood samples.
- Standard precautionary instructions must be followed by all healthcare professionals during the collection and transportation of whole blood samples.
- Whole blood samples should be collected in appropriate containers before delivery to the laboratory.
- Freezing and thawing of samples should be avoided.

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

DNA EXTRACTION

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.
Our system optimized according to GeneAll® Exgene™ Blood SV. It is advised to elute DNA with 150 µl elution buffer for better results.

PROCEDURE

- Different test tubes should be prepared for each master mix.
- Leave the master mixes* and controls at RT to melt.
- Before starting work, mix the master mixes gently by pipetting
- For each sample, pipet **20 µl master mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube. Please do not pipette DNA before and after addition into well.
- Optical caps are closed, it is recommended to spin the plates/strips at low speed for a short time.
- Run with the programme shown below.

*Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Table 2: PCR Programme

Fluorescent dyes are FAM and HEX/JOE.

<u>This system can be used with the following devices;</u>
- Bio-Rad CFX96
- ABI Prism ® 7500/7500 Fast
- Roche LightCycler® 480 System
- Rotor Gene Q
- Mic qPCR Cycler

For other two or more channel Real-Time PCR devices (which can read FAM and HEX/JOE dyes), a trial run is recommended.

<u>If you use;</u>
ABI Prism® system, please choose **"none"** as passive reference and quencher.
Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10**.

Supplied Materials
- White PCR plates/strips with optical covers*

*The PCR Plate/strip tube and caps seriously affect the amplification curve quality. Therefore, white PCR plates/strips and optical caps provided by the manufacturer should be used with the kit.

Required Materials (Not Provided)
- PCR Cabinet
- Vortex Mixer
- Desktop Microcentrifuge (For 2.0ml tubes and PCR strip tubes), plate spin for studies using PCR plates.
- Automated or spin column based DNA isolation Kit
- Disposible powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

CE IVD

SNP Biotechnology R&D Ltd.
Hacettepe Technopolis 1. R&D Building Office No: 10 Beytepe – ANKARA / TURKEY
Tel : +90312-2992328 / Fax : +90312-2992348 / www.snp.com.tr / info@snp.com.tr

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX/JOE and FAM dyes. The below results were studied with Bio-Rad CFX96. The threshold values for both FAM and HEX/JOE dyes were set to 500, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system, the GeneAll® Exgene™ Blood SV Isolation Kit, and white PCR strips supplied by SNP Biotechnology. Threshold values may vary depending on the PCR device, DNA isolation kit, and the type or brand of PCR strips/tubes used.

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The C_T value of internal controls should be **$20 \leq C_T \leq 28$**. These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device. C_T values may vary ±2/3 cycle according to the other DNA isolation systems and Real-Time PCR devices (Figure 1).

Amplification plots of HLA-B52 can be analysed by FAM dye. . The C_T values of HLA-B52 Positive sample should be **$20 \leq C_T \leq 28$**. These values are optimised according to the GeneAll® Exgene™ Blood SV Isolation Kit and Bio-Rad CFX96 Real-Time PCR Device. C_T values may vary ±2/3 cycle according to the other DNA isolation systems and Real-Time PCR devices The two master mixes must be give plot for **positive** result (Figure 2). If only one mix give a plot or if neither, the result is negative (Figure 3-5).

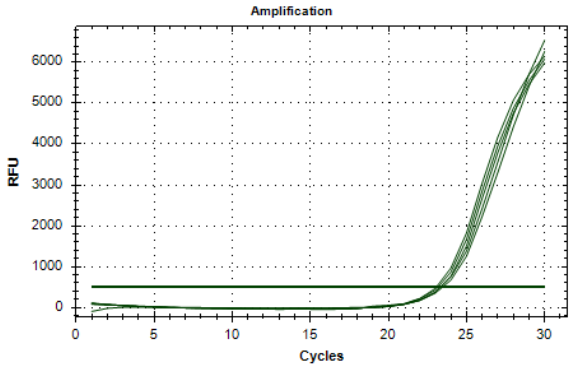

Figure 1: Internal Control plots – HEX/JOE Dye

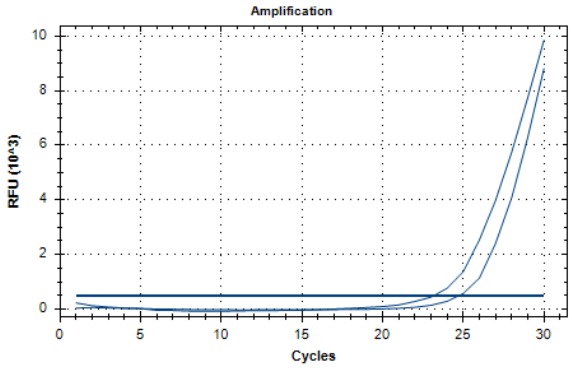

Figure 2: HLA-B52 Positive Sample (FAM Dye)

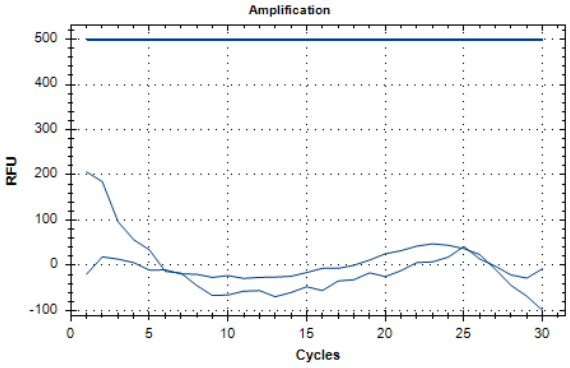

Figure 3 : HLA-B52 Negative Sample (FAM Dye)

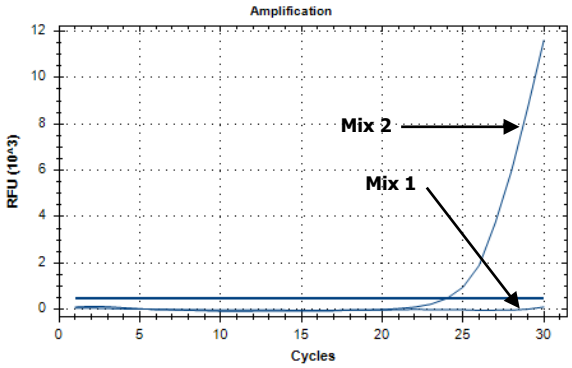

Figure 4 : HLA-B52 Negative Sample (FAM Dye)

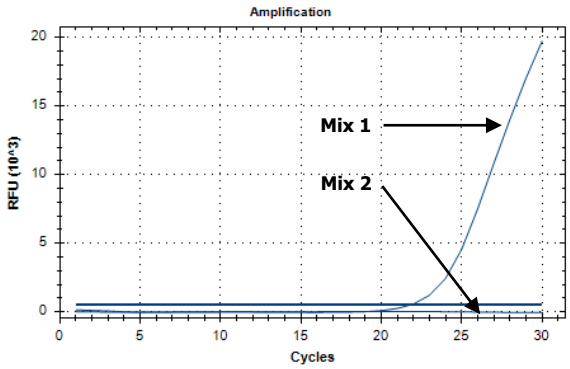

Figure 5 : HLA-B52 Negative Sample (FAM Dye)

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR master mixes forgotten at room temperature.
- Thaw PCR master mix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR master mix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

DISPOSAL OF KIT

Dispose of it according to the legal regulations of your region

CE IVD

SNP Biotechnology R&D Ltd.
Hacettepe Technopolis 1. R&D Building Office No: 10 Beytepe – ANKARA / TURKEY
Tel : +90312-2992328 / Fax : +90312-2992348 / www.snp.com.tr / info@snp.com.tr

SYMBOLS AND DESCRIPTIONS

REF	Catalog Number	CE	CE Mark
LOT	Lot Number	UDI	Unique Device Identifier (01)Device Identifier (17)Expiry Date (10)Lot Number
	Manufacturer	Σ	Test Quantity
	Fragile	-20 °C	Storage Temperature
	Protect from directly sunlight	IVD	In Vitro Diagnostics
	Expiry Date		

Table 3: Symbols and descriptions

REFERENCES

1. Mathilde De Menthon, Michael P. Lavalley, Carla Maldini, Loïc Guillevin, and Alfred Mahr. "HLA–B51/B5 and the Risk of Behçet's Disease: A Systematic Review and Meta-Analysis of Case–Control Genetic Association Studies". Arthritis Rheum. 2009 Oct 15; 61(10): 10.1002/art.24642.
2. Kota Sugisaki, Rie Saito, Tadayuki Takagi, Kiori Shio, Yasuo Shioya, Etsuko Fukaya, Haruyo Iwadate, Hideharu Sekine, Hiroshi Orikasa, Hiroko Kobayashi, Hiroshi Watanabe and Yukio Sato. "HLA-B52-positive vasculo-Behçet disease: usefulness of magnetic resonance angiography, ultrasound study, and computed tomographic angiography for the early evaluation of multiarterial lesions". Mod Rheumatol (2005) 15:56–61.
3. https://www.ebi.ac.uk/ipd/imgt/hla/
4. Yolanda S Lie and Christos J Petropoulos. "Advances in quantitative PCR technology: 5' nuclease assays". Current Opinion in Biotechnology Volume 9, Issue 1, February 1998, Pages 43-48.
5. Luis Ugozzoli and R. Bruce Wallace. "Allele-Specific Polymerase Chain Reaction". A Companion to Methods in Enzymology Vol. 2, No. 1, February, pp. 42-48, 1991.

TROUBLESHOOTING PROBLEMS AND SOLUTIONS

Problem	Reason	Solution
Internal control does not work/ low amplification	Absence of DNA / DNA extraction problems	Repeat test
	Absence of DNA / DNA extraction problems	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
	Sample is containing PCR inhibitor(s)	
No target DNA/internal control amplification curves in all wells	Error in temperature/time settings in PCR program	Correct any errors in the temperature/time settings in the PCR Program and repeat the test.
	Sample is containing PCR inhibitor(s)	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
Positive control result and/or C_T values are lower or higher than the value mentioned in User Manual.	Error in temperature/time settings in PCR program	Correct any errors in the temperature/time settings in the PCR Program and repeat the test.
C_T values are not valid (higher or lower) according to User Manual	Excessive or insufficient DNA sample	• Repeat the test. • DNA extraction should be repeated.
Low and/or invalid amplification curves	Stability problems arising from repeated thawing and freezing (>4X)	Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.
	Sample is containing PCR inhibitor(s)	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
	Stability problems arising from unavailable storage conditions.	All reagents should be stored at – 20 °C and dark.
	Bubble formation or pipetting error during pipetting	After adding the master mix and sample, it is recommended to spin the plates/strips at low speed for a short time.
For further questions, please contact us **tech@snp.com.tr**		

Table 4: Troubleshooting problems and solutions