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)]. The kit detect all subtypes of HLA B52 in the **IMGT / HLA Gene FASTA 3.32.0 database** with high specificity.

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 (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

The kit provides reagents in a **"ready-to-use"** master mix format which has been specifically adapted for 5' nuclease PCR. The test system is designed for use with sequence specific primers and probe.

The fluorescence of HLA B52 analysis is FAM in Master Mix 1 and Master Mix 2. Also each master mix contains an internal control labelled with HEX/JOE dye.

HEX/JOE dye.

The limit of detection (LOD) in HLA B52 Kit was determined as 1 ng/µl.

SYSTEM CONTENTS

Reagents	20 rxns
• HLA B52 Mix 1	400 µl
• HLA B52 Mix 2	400 µl
• Positive Control DNA	30 µl
• Negative Control DNA	30 µl

* Control DNAs contain plasmid and amplification plots of plasmid DNAs may differ slightly from sample DNA plots.

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 SNPure Blood® and MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

PROCEDURE

- Different Mix 1 and Mix 2 tubes should be prepared.
- Before starting work, mix the mastermixes gently by pipetting.
- For each sample, pipet **20 µl mastermix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube**.**
- Mix gently by pipetting
- Run with the programme shown below.

PCR PROGRAMME

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

Fluorescent dyes are FAM and HEX/JOE.

If you use;

- 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.**

This system can use with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

Roche LightCycler® 480 System

Rotor Gene Q

Mic qPCR Cycler

DATA ANALYSIS

The run is completed and data are analysed using the software with HEX and FAM dyes. The below results were studied with Bio-Rad CFX96.

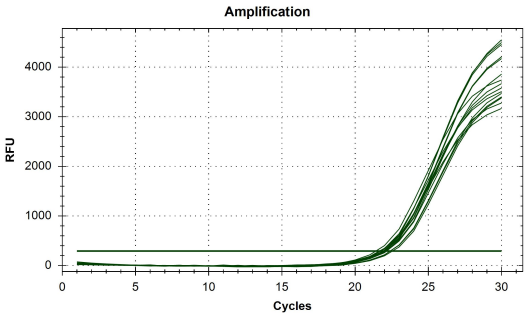

Figure 1: Internal Control Plots (HEX/JOE Dye)

Internal control amplification plots must be seen in all wells except NTC and it has been labelled with HEX/JOE dye. The CT value of internal controls should be **20 ≤ X ≤ 26** (Figure 1).

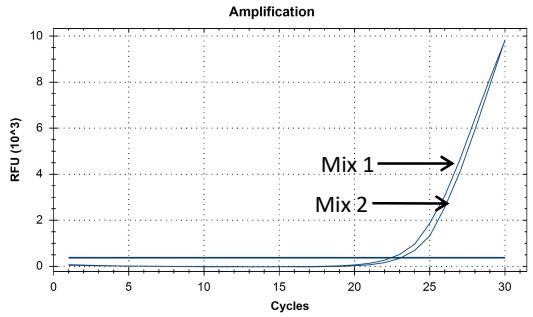

Figure 2 : HLA B52 Positive sample - Mix 1 and Mix 2 (FAM Dye)

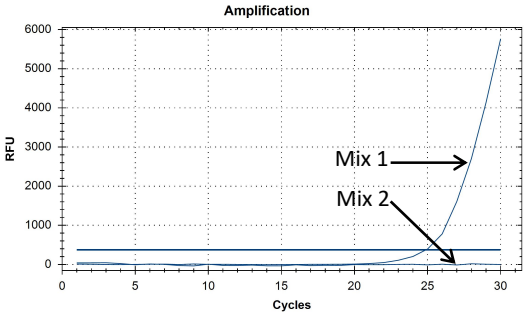

Figure 3 : HLA B52 Negative sample – Only Mix 1 positive* (FAM Dye)

*** The two master mixes must be give plot for positive result. If only one mix give a plot or if neither, the result is negative.**

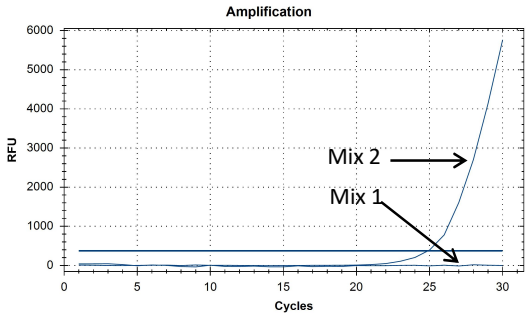

Figure 4 : HLA B52 Negative sample – Only Mix 2 positive* (FAM Dye)

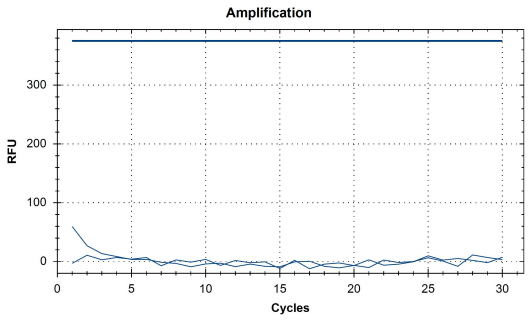

Figure 5 : HLA B52 Negative sample – Both mixes are negative* (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

- DNA quality is not good.
- The amount of DNA is not enough.

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

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

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. Jae Kyoun Ahn and Yeoung Geol Park. "Human Leukocyte Antigen B27 and B51 Double-Positive Behçet Uveitis."Arch Ophthalmol. 2007;125(10):1375-1380. doi:10.1001/archopht.125.10.1375.