HLA B57 REAL TIME PCR KIT

Cat. No: 505R-10-01

INTRODUCTION

International HIV treatment guidelines recommend HLA-B*57:01 typing before abacavir administration, in order to reduce the incidence of abacavir hypersensitivity reactions, the major cause of early therapy discontinuation. [1,2]. The kit detect all subtypes of HLA B57 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

Each isolated DNA should be tested with HLA B57 Master Mix. 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 B57 analysis is FAM. Also each mastermix contains an internal control labelled with HEX/JOE dye.

The limit of detection (LOD) in HLA B57 Kit was determined as 1 ng/µl.

SYSTEM CONTENTS

Reagents	20 rxns
• HLA B57 Master Mix	400 µl
• Positive Control DNA	30 µl
• Negative Control DNA	30 µl

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

- 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**.**
- 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

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

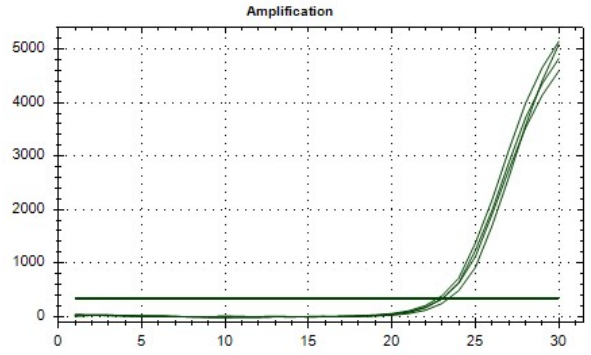

Figure 1: Internal Control Plots (HEX/JOE Dye)

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

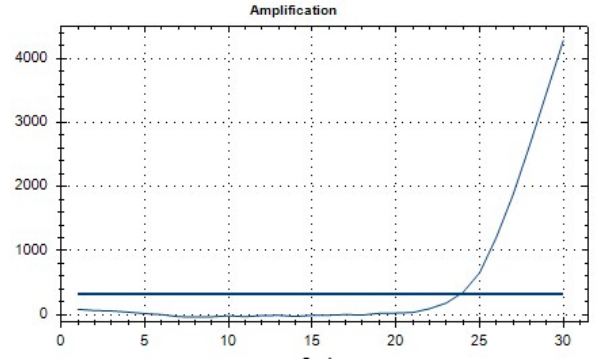

Figure 2 : HLA B57 Master Mix – Positive sample (FAM Dye)

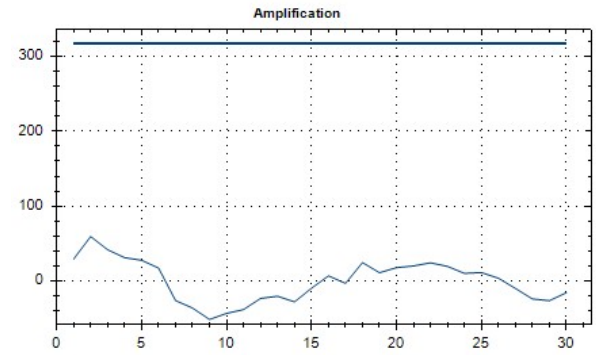

Figure 3 : HLA B57 Master Mix – Negative sample (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work,

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

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- 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. Cinzia Dello Russo†, Lucia Lisi, Alessia Lofaro, Simona Di Giambenedetto, Bruno Federico, Giordano Madeddu, Marianna Salerno, Maria Stella Mura, Antonella Pirazzoli, Andrea de Luca, Roberto Cauda and Pierluigi Navarra. Novel sensitive, specific and rapid pharmacogenomic test for the prediction of abacavir hypersensitivity reaction: HLA-B*57:01 detection by real-time PCR. Pharmacogenomics (2011) 12(4), 567–576.
2. A.M. Martin, D. Nolan, S. Mallal. HLA-B*5701 typing by sequence-specific amplification: validation and comparison with sequence-based typing. Tissue Antigens 2005: 65: 571–574.