HLA B27 REAL TIME PCR KIT

Cat. No: 501R-10-01

INTRODUCTION

Human leukocyte antigen (HLA) B27 is a class I surface antigen encoded by the B locus in the major histocompatibility complex (MHC) on chromosome 6. HLA-B27 is associated with ankylosing spondylitis (AS), and other associated inflammatory diseases referred to as "spondyloarthritis" [1,2]. The kit detect all subtypes of HLA B27 in the **IMGT / HLA Gene FASTA 3.32.0 database** with high specificity except B27:07:01, B27:07:04, B27:24, B27:32 and B27:70 (See table 1).

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 B27 Master Mix. The kit provides reagents in a **"ready-to-use"** mastermix 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 B27 analysis is FAM. Also each mastermix contains an internal control labelled with HEX/JOE dye.

The limit of detection (LOD) in HLA B27 Kit was determined as 1 ng/µl.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxns
• HLA B27 Master Mix	400 µl	1000 µl
• Positive Control DNA	20 µl	40 µl
• Negative Control DNA	20 µl	40 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

- 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**.**
- Optical caps are closed and 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, Opus 96

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. 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 control amplification ct values should be **20 ≤ X ≤ 26** at HEX dye (Figure 1). The presence of amplification plots at the FAM dye should be evaluated as **"HLA B27 Positive".** Amplification ct values should be **22 ≤ X ≤ 27** for positive DNA samples and positive control at FAM dye. These ranges may differ depending on the PCR instrument, threshold values and tubes used. For positive samples; Amplification plots of internal control and HLA B27 should be close to each other and not exceed 4 Ct (Figure 2 and 3).

If there is no amplification plot at the FAM dye, the sample is **"HLA B27 negative"** (Figure 4).

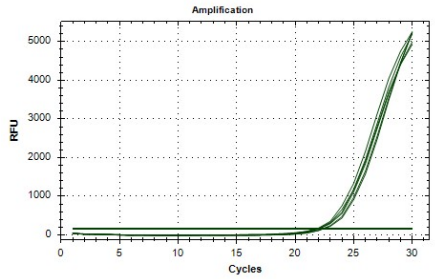

Figure 1: Internal Control Plots (HEX/JOE Dye)

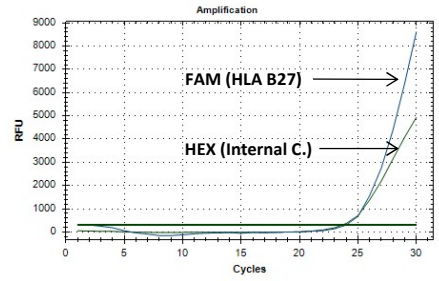

Figure 2: HLA B27 Master Mix – Positive control (FAM and HEX Dye)

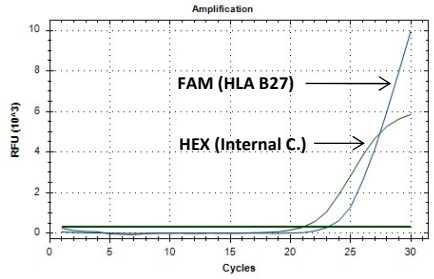

Figure 3: HLA B27 Master Mix – Positive DNA Sample (FAM and HEX Dye)

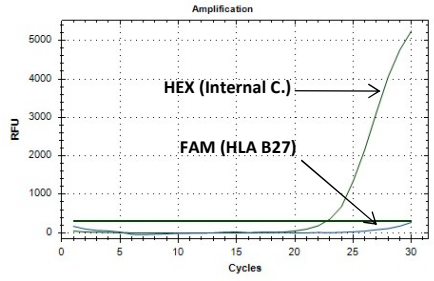

Figure 4: HLA B27 Master Mix – Negative DNA sample (FAM and HEX Dye)

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.

Table 1: List of B27 subtypes detected with the kit.

Subtypes of HLA B27			
B*27:01	B*27:05:18:01	B*27:118	B*27:207
B*27:02:01:01	B*27:05:18:02	B*27:123	B*27:208
B*27:02:01:02	B*27:05:23	B*27:131	B*27:209
B*27:02:01:03	B*27:05:31	B*27:137	B*27:210
B*27:02:01:04	B*27:05:32	B*27:142	B*27:211
B*27:02:01:05	B*27:05:33	B*27:144:01	B*27:212N
B*27:02:01:06	B*27:05:34	B*27:146	B*27:213
B*27:02:01:07	B*27:05:35	B*27:150	B*27:214
B*27:02:01:08	B*27:05:36	B*27:157	B*27:216
B*27:02:05	B*27:05:37	B*27:158	B*27:217
B*27:02:06	B*27:05:39	B*27:162	B*27:218
B*27:03	B*27:05:40	B*27:163	B*27:219
B*27:04:01	B*27:05:41	B*27:165	B*27:220
B*27:05:02:01	B*27:05:42	B*27:169	B*27:221
B*27:05:02:02	B*27:05:43	B*27:170	B*27:222
B*27:05:02:03	B*27:05:44	B*27:173	B*27:223N
B*27:05:02:04Q	B*27:05:45	B*27:174	B*27:224
B*27:05:02:05	B*27:05:46	B*27:175	B*27:227
B*27:05:02:06	B*27:05:48	B*27:176N	B*27:229
B*27:05:02:07	B*27:05:49	B*27:177	B*27:230
B*27:05:02:08	B*27:05:5	B*27:178:01	B*27:231
B*27:05:02:09	B*27:05:51	B*27:178:02	B*27:232
B*27:05:02:10	B*27:05:52	B*27:179	B*27:233
B*27:05:02:11	B*27:05:53	B*27:180	B*27:234
B*27:05:02:12	B*27:05:54	B*27:181	B*27:235
B*27:05:02:13	B*27:05:55	B*27:182	B*27:236
B*27:05:02:14	B*27:05:56	B*27:184	B*27:237
B*27:05:02:15	B*27:06:01:01	B*27:185Q	B*27:238
B*27:05:02:16	B*27:06:01:02	B*27:186	B*27:239
B*27:05:02:17	B*27:08	B*27:187	B*27:240
B*27:05:02:18	B*27:09	B*27:188	B*27:241
B*27:05:02:19	B*27:10	B*27:189	B*27:242
B*27:05:02:20	B*27:12:01:01	B*27:190	B*27:243N
B*27:05:02:21	B*27:12:01:02	B*27:191	B*27:244
B*27:05:02:22	B*27:12:01:03	B*27:192	B*27:245
B*27:05:02:23	B*27:13:01	B*27:193	B*27:246N
B*27:05:02:24	B*27:14	B*27:194	B*27:247
B*27:05:02:25	B*27:15	B*27:195	B*27:248
B*27:05:02:26	B*27:17	B*27:196	B*27:249
B*27:05:02:27	B*27:19:01:01	B*27:197	B*27:250
B*27:05:02:28	B*27:19:01:02	B*27:200	B*27:251
B*27:05:02:29	B*27:20	B*27:201	B*27:252
B*27:05:02:30	B*27:21:02	B*27:202	B*27:253Q
B*27:05:02:31	B*27:25	B*27:203	B*27:254N
B*27:05:02:32	B*27:30	B*27:204:01:01	B*27:255
B*27:05:03	B*27:90:04	B*27:204:01:02	
B*27:05:05	B*27:91	B*27:205	
B*27:05:07	B*27:101	B*27:206	

REFERENCES

1. Danilo Garcia Ruiz, Mário Newton Leitão de Azevedo and Omar Lupi. "HLA-B27 frequency in a group of patients with psoriatic arthritis". An Bras Dermatol. 2012;87(6):847-50.
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.