HLA B27 REAL TIME PCR KIT WITH DNA EXTRACTION

Cat. No: 501R-10-02

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
• Solution E	1000 µl x2	1700 µl x3
• 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

- Keep the Solution E to melt at room temperature before starting to work. After the first use, the **Solution E** can be stored at Room Temperature.
- Mix by inverting the **Solution E** tube and transfer **100 µl** into 1.5 microcentrifuge tube.
- Add **100 µl whole blood** tube and pipetting 3-4 times gently (Mix by inverting the peripheral blood tubes before adding blood).
- Incubate at **92 °C for 15 minutes.**
- Centrifuge at **10.000 rpm for 3** minutes.
- Use 5 µl of supernatant as a PCR template.
- We recommend use peripheral blood tubes stored at +4 to 8 °C and fresh extraction for the test.

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.	35-40 Cycles *
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE.

***The number of cycles may vary depending on the PCR instrument and tubes used.**

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

Long Gene

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 controls and FAM dye for positive samples should be **23 ≤ X ≤ 33** (Figure 1-2). These ranges may differ depending on the PCR instrument and tubes used.

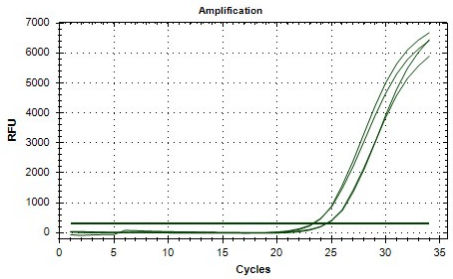

Figure 1: Internal Control Plots (HEX/JOE Dye)

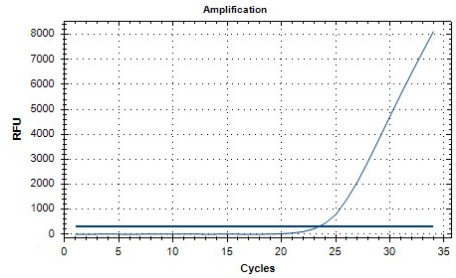

Figure 2 : HLA B27 Master Mix – Positive sample (FAM Dye)

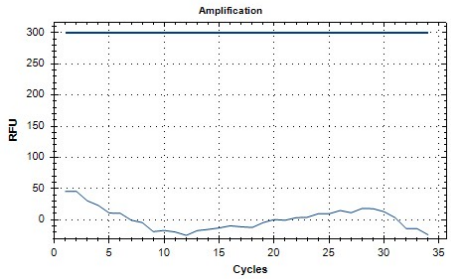

Figure 3 : HLA B27 Master Mix – Negative sample (FAM Dye)

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.

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.