DIRECT HLA B27 REAL TIME PCR KIT

Cat. No: 501R-10-03

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 can directly detect HLA B27 from whole blood samples without DNA extraction step. The kit analyzes 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 sample should be tested with HLA B27 Direct Mix. The kit provides reagents in a **"ready-to-use"** Direct 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 B27 analysis is FAM. Also each Direct Mix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	20 rxns	50 rxns
• HLA B27 Direct Mix	400 µl	1000 µl
• Positive Control *	20 µl	40 µl
• Negative Control *	20 µl	40 µl

* Controls contain plasmid and amplification plots of controls may differ slightly from whole blood sample 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

The Direct Kit System is suitable to work from whole blood without DNA extraction step.

PROCEDURE

- Leave the Direct mix and controls at RT to melt.
- Mix the melted Direct mix gently by pipetting.
- For each sample, pipet **20 µl Direct mix** with micropipets of sterile filter tips into **White PCR tubes / plate.**
- Turn the peripheral blood tubes for mixing.
- Add **0.5 µl whole blood** into each **White PCR tubes / plate.**
- Optical caps are closed and run with the programme shown below.

PCR PROGRAMME

95 °C	5 Min.	Holding
95 °C	10 Sec.	40 Cycles *
63°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 control and HLA B27 amplification should be **25 ≤ X ≤ 32** (Figure 1). These ranges may differ depending on the PCR instrument and tubes used. The presence of amplification plots at the FAM dye should be evaluated as HLA B27 Positive. For positive samples; Amplification plots of internal control and HLA B27 should be close to each other and not exceed 4 Ct (Figure 2).

If there is no amplification plot at the FAM dye, the sample is HLA B27 negative (Figure 3).

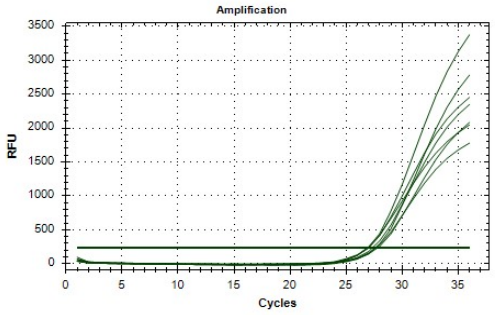

Figure 1: Internal Control Plots (HEX/JOE Dye)

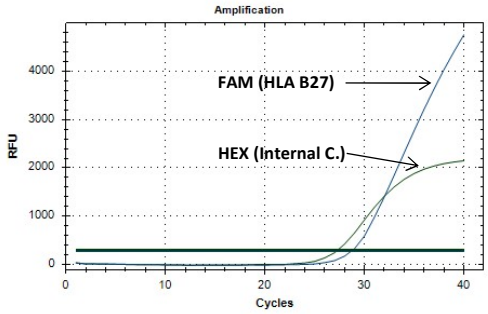

Figure 2 : HLA B27 Direct Mix – Positive sample (FAM and HEX Dyes)

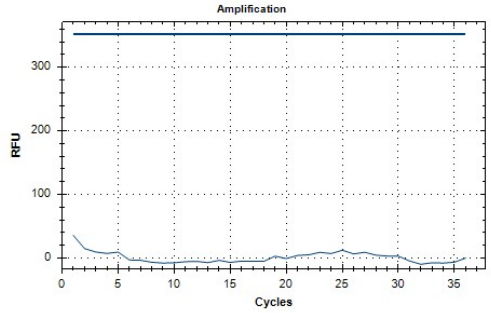

Figure 3 : HLA B27 Direct Mix – Negative sample (FAM Dye)

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of sample
- Sample is containing serious PCR inhibitor(s)

Please re-test the sample.

For further questions, please contact us tech@snp.com.tr .

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR Direct mix forgotten at room temperature.
- Thaw PCR Direct mix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR Direct mix 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.