

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).

INTENDED USE

Direct HLA-B27 Real-Time PCR Kit can detect subtypes of 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 5).

TARGETED USER

For professional use only. Testing should be performed by professionals trained in molecular techniques.

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 quencher 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 (C_T) is proportional to the amount of the specific PCR product (3,4).

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 to 5' nuclease PCR for SNP analysis. The test system is designed by SNP Biotechnology for use with sequence specific primers and probes.

The fluorescence of HLA-B27 subtypes analysis is FAM. Also Direct Mix contains an internal control labelled with HEX/JOE dye. Internal Control is Prothrombin gene – FII (OMIM: 176930).

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
HLA-B27 Direct Mix	200 µl	400 µl	1000 µl
Positive Control*	30 µl	30 µl	60 µl
Negative Control*	30 µl	30 µl	60 µl

Table 1: Kit content

*Since to Controls are synthetic plasmids, amplification plots of synthetic controls may appear slightly different from the sample. Please gently vortex and then spin centrifuge for 1-2 seconds before use the controls.

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.

SAMPLE COLLECTION

Direct HLA-B27 Real-Time PCR Kit is approved for use with whole blood samples.

- Standard precautionary instructions must be followed by all healthcare professionals during the collection and transportation of whole blood samples.
- Whole blood samples should be collected in appropriate containers before delivery to the laboratory.
- Freezing and thawing of samples should be avoided.

DNA EXTRACTION

Direct HLA-B27 Real-Time PCR Kit System is suitable to work from whole blood without DNA extraction step.

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.

PROCEDURE

- Leave the Direct mix* and controls at RT to melt.
- Before starting work, mix the direct mix gently by pipetting
- For each sample, pipet **20 µl Direct mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **0.5 µl whole blood** into each tube. Please do not pipette blood before and after addition into well.
- Optical caps are closed, it is recommended to spin the plates/strips at low speed for a short time.
- Run with the programme shown below.

*Direct mix include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	5 Min.	Holding
95 °C	10 Sec.	35-40 Cycles*
63 °C	1 Min.	

Table 2: PCR Programme

Fluorescent dyes are FAM and HEX/JOE.

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

This system can be used with the following devices:

- Bio-Rad CFX96
- ABI Prism® 7500/7500 Fast
- Roche LightCycler® 480 System
- Rotor Gene Q
- Mic qPCR Cycler

For other two or more channel Real-Time PCR devices (which can read FAM and HEX/JOE dyes), a trial run is recommended.

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.

Supplied Materials

- White PCR plates/strips with optical covers*

*The PCR Plate/strip tube and caps seriously affect the amplification curve quality. Therefore, white PCR plates/strips and optical caps provided by the manufacturer should be used with the kit.

Required Materials (Not Provided)

- PCR Cabinet
- Vortex Mixer
- Desktop Microcentrifuge (For 2.0ml tubes and PCR strip tubes), plate spin for studies using PCR plates.
- Disposable powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX/JOE and FAM dyes. The below results were studied with Bio-Rad CFX96. The threshold values for both FAM and HEX/JOE dyes were set to 300, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system and white PCR strips supplied by SNP Biotechnology. Threshold values may vary depending on the PCR device and the type or brand of PCR strips/tubes used.

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The C_T value of internal controls should be $25 \leq C_T \leq 34$. These values are optimised according to Bio-Rad CFX96 Real-Time PCR Device and white PCR strips supplied by SNP Biotechnology. C_T values may vary $\pm 2/3$ cycle according to the other tubes and Real-Time PCR devices (Figure 1).

The presence of amplification plots at the FAM dye should be evaluated as **"HLA-B27 Positive"**. Amplification C_T values should be $25 \leq C_T \leq 34$ for positive 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 overlapped or close to each other and C_T differences should not exceed 2 (Figure 2).

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

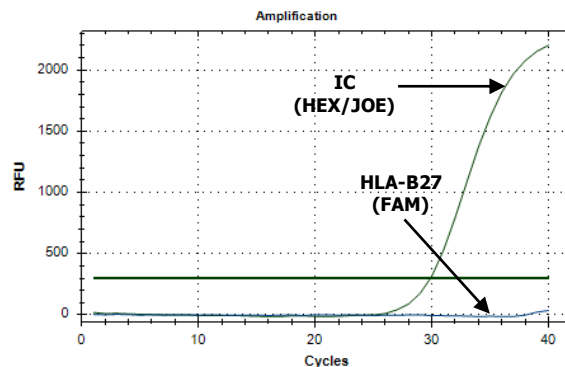


Figure 3: HLA-B27 Negative Sample (FAM and HEX/JOE Dyes)

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.

DISPOSAL OF KIT

Dispose of it according to the legal regulations of your region

SYMBOLS AND DESCRIPTIONS












	Catalog Number		CE Mark
	Lot Number		Unique Device Identifier (01)Device Identifier (17)Expiry Date (10)Lot Number
	Manufacturer		Test Quantity
	Fragile		Storage Temperature
	Protect from directly sunlight		In Vitro Diagnostics
	Expiry Date		

Table 3: Symbols and descriptions

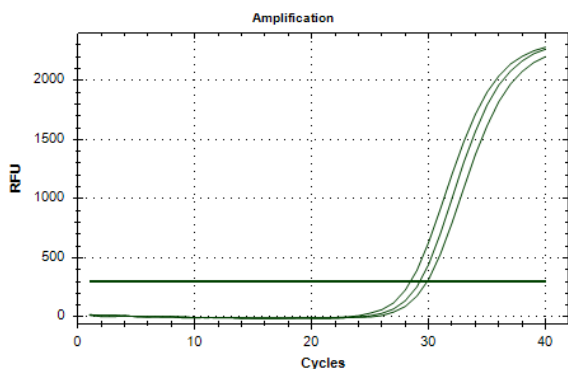


Figure 1: Internal Control plots – HEX/JOE Dye

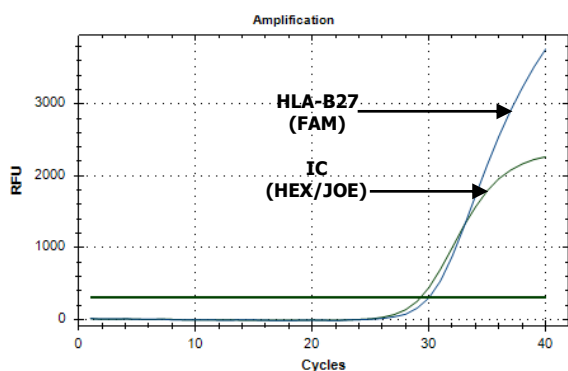


Figure 2 : HLA-B27 Positive Sample (FAM and HEX/JOE Dyes)

TROUBLESHOOTING PROBLEMS AND SOLUTIONS

Problem	Reason	Solution
Internal control does not work/ low amplification	Absence of Sample / not added into well	Repeat test
	Sample is containing PCR inhibitor(s)	
No target /internal control amplification curves in all wells	Error in temperature/time settings in PCR program	Correct any errors in the temperature/time settings in the PCR Program and repeat the test.
	Sample is containing PCR inhibitor(s)	Repeat test
Positive control result and/or C _T values are lower or higher than the value mentioned in User Manual.	Error in temperature/time settings in PCR program	Correct any errors in the temperature/time settings in the PCR Program and repeat the test.
C _T values are not valid (higher or lower) according to User Manual	Excessive or insufficient sample	Repeat the test.
Low and/or invalid amplification curves	Stability problems arising from repeated thawing and freezing (>4X)	Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.
	Sample is containing PCR inhibitor(s)	Repeat the test.
	Stability problems arising from unavailable storage conditions.	All reagents should be stored at – 20 °C and dark.
	Bubble formation or pipetting error during pipetting	After adding the Direct mix and sample, it is recommended to spin the plates/strips at low speed for a short time.
For further questions, please contact us tech@snp.com.tr		

Table 4: Troubleshooting problems and solutions

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

Table 5: List of HLA-B27 subtypes detected with the kit.

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 Kyoung 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/archophth.125.10.1375.
3. Yolanda S Lie and Christos J Petropoulos. "Advances in quantitative PCR technology: 5' nuclease assays". Current Opinion in Biotechnology Volume 9, Issue 1, February 1998, Pages 43-48.
4. Luis Ugozzoli and R. Bruce Wallace. "Allele-Specific Polymerase Chain Reaction". A Companion to Methods in Enzymology Vol. 2, No. 1, February, pp. 42-48, 1991.