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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].

INTENDED USE
HLA B27 Real-Time PCR Kit With DNA Extraction can 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 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 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 (C_T) is proportional to the amount of the specific PCR product [3,4].

PRODUCT SPECIFICATION
Each sample should be tested with HLA B27 Master mix. The kit provides reagents in a **"ready-to-use"** master 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 analysis is FAM. Also each Master Mix contains an internal control labelled with HEX/JOE dye. Internal Control is Prothrombin gene – FII (OMIM: 176930).
The limit of detection (LOD) for the HLA B27 Real-Time PCR Kit With DNA Extraction was determined as 1 ng/µl.

SYSTEM CONTENTS

Reagents	10 rxns	20 rxns	50 rxns
HLA B27 Master Mix	200 µl	400 µl	1000 µl
Solution E	1000 ul x1	1000 ul x2	1700 ul x3
Positive Control*	30 µl	30 µl	60 µl
Negative Control*	30 µl	30 µl	60 µl

Table 1: Kit content
*Since to Control DNAs are synthetic plasmids, amplification plots of synthetic control DNAs may appear slightly different from the sample DNA. 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
HLA B27 Real-Time PCR Kit With DNA Extraction 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
- 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
- Leave the master mix* and controls at RT to melt.
- Before starting work, mix the master mix gently by pipetting
- For each sample, pipet **20 µl master mix** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube. Please do not pipette DNA 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.

*Master mix include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	35-40 Cycles*
60 °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.**

<u>This system can be used with the following devices;</u>
- 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.

<u>If you use;</u>
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.
- Automated or spin column based DNA isolation Kit
- Disposible powder-free laboratory gloves
- Micropipettes (0.5ml-1000ml)
- Micropipette tips
- Standard laboratory equipments.

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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 1000, based on experiments conducted using the Bio-Rad CFX96 Real-Time PCR system, Solution E Isolation, 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 **$23 \leq C_T \leq 33$**. These values are optimised according to the Bio-Rad CFX96 Real-Time PCR system, Solution E Isolation, and white PCR strips supplied by SNP Biotechnology. C_T values may vary ±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 **$23 \leq C_T \leq 33$** 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 (HEX/JOE) and HLA B27 (FAM) should be 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).

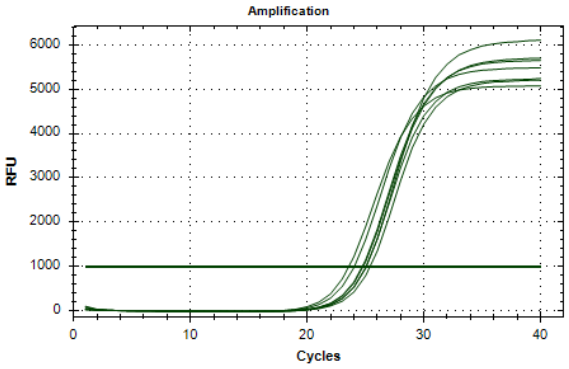

Figure 1: Internal Control plots – HEX/JOE Dye

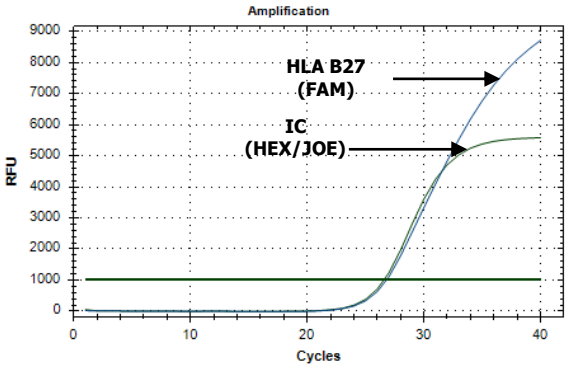

Figure 2 : HLA B27 Positive Sample (FAM and HEX/JOE Dyes)

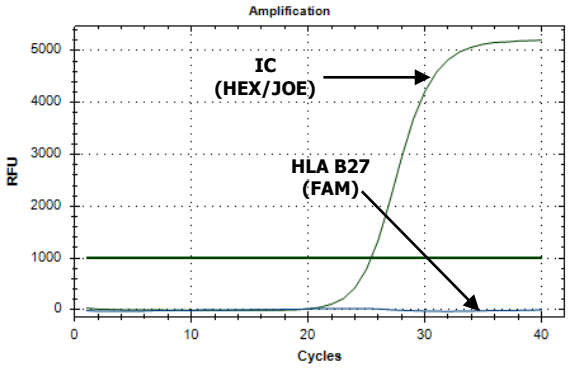

Figure 3: HLA B27 Negative Sample (FAM and HEX/JOE Dyes)

CAUTIONS

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

REF	Catalog Number	CE	CE Mark
LOT	Lot Number	UDI	Unique Device Identifier (01)Device Identifier (17)Expiry Date (10)Lot Number
	Manufacturer	Σ	Test Quantity
	Fragile	-20 °C	Storage Temperature
	Protect from directly sunlight	IVD	In Vitro Diagnostics
	Expiry Date		

Table 3: Symbols and descriptions

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TROUBLESHOOTING PROBLEMS AND SOLUTIONS

Problem	Reason	Solution
Internal control does not work/ low amplification	Absence of DNA / DNA extraction problems	Repeat test
	Absence of DNA / DNA extraction problems	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
	Sample is containing PCR inhibitor(s)	
No target DNA/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)	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
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 DNA sample	• Repeat the test. • DNA extraction should be repeated.
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)	• DNA extraction should be repeated. • DNA extraction should be replaced with one of the recommended methods.
	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 master 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

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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 B27 subtypes detected with the kit.

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