



OCTO Health RT-PCR HLA-B27 GENOTYPING KIT

Catalog

RT-HLA-B27-25

RT-HLA-B27-50

Description

Real-Time PCR Human Leukocyte Antigen Genotyping Kit, 25 reactions

Real-Time PCR Human Leukocyte Antigen Genotyping Kit, 50 reactions

IVD



Introduction

HLA-B27 is a protein belonging to the MHC class I family, responsible for antigen presentation in the immune system. This genetic variant is notable for its association with a predisposition to autoimmune diseases. HLA-B27 has a strong link to diseases such as ankylosing spondylitis and reactive arthritis, in which the immune system may mistakenly attack its own tissues. Found at varying frequencies across different populations, HLA-B27 consists of several sub-variants with unique amino acid sequences. These sub-variants carry different levels of risk for disease development and can influence the likelihood of certain autoimmune conditions in individuals carrying HLA-B27.

Diagnosis

HLA-B27 diagnosis is typically made through genetic analyses combined with clinical symptoms and laboratory tests. Tests performed using the Real-Time PCR Kit provide a precise and rapid method for determining the presence of the HLA-B27 allele. This method amplifies DNA from a blood sample and targets specific sequences of the HLA-B27 gene. A positive result may indicate the patient's predisposition to certain autoimmune diseases, such as ankylosing spondylitis and reactive arthritis. However, the presence of the HLA-B27 allele alone is not sufficient for a diagnosis; doctors must conduct a comprehensive evaluation considering the patient's medical history, physical examination findings, and other laboratory results. The rapid and reliable results provided by the Real-Time PCR test enhance the efficiency of the diagnostic process.

OCTO Health REAL TIME PCR HLA-B27 Genotyping Kit is based on fluorescent modification of the PCR method. The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

Technical Specifications

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit. The Real-Time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target-specific probes for detecting the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for HLA-B27 assays are labelled with the fluorophore FAM, while the probe specific for the Internal Control (IC) is labelled with the fluorophore HEX.

This setup ensures accurate detection of HLA-B27 Assays and simultaneous verification of the test integrity through the Internal Control in a real-time PCR assay.

Kit Contents

Component	Content	25 Reactions	50 Reactions
1	HLA-B27 Master Mix 1	450 µl	900 µl
	Internal Control	Included	
2	Positive Control	25µL	50µL
3	Negative Control	50 µL	100µL

COVERED HLA-B27 SUBTYPES (330 SUBTYPES)

B*27:01,B*27:02:01:01,B*27:02:01:02,B*27:02:01:03,B*27:02:01:04,B*27:02:01:05,B*27:02:01:06,B*27:02:01:07,B*27:02:01:08,B*27:02:01:09,B*27:02:02,B*27:02:03,B*27:02:04,B*27:02:05,B*27:02:06,B*27:02:07,B*27:03,B*27:04:01,B*27:04:02,B*27:04:03,B*27:04:04,B*27:04:05,B*27:04:06,B*27:05:02:01,B*27:05:02:02,B*27:05:02:03,B*27:05:02:04Q,B*27:05:02:05,B*27:05:02:06,B*27:05:02:07,B*27:05:02:08,B*27:05:02:09,B*27:05:02:10,B*27:05:02:11,B*27:05:02:12,B*27:05:02:13,B*27:05:02:14,B*27:05:02:15,B*27:05:02:16,B*27:05:02:17,B*27:05:02:18,B*27:05:02:19,B*27:05:02:20,B*27:05:02:21,B*27:05:02:22,B*27:05:02:23,B*27:05:02:24,B*27:05:02:25,B*27:05:02:26,B*27:05:02:27,B*27:05:02:28,B*27:05:02:29,B*27:05:02:30,B*27:05:02:31,B*27:05:02:32,B*27:05:02:33,B*27:05:02:34,B*27:05:02:35,B*27:05:02:36,B*27:05:02:37,B*27:05:02:38,B*27:05:02:39,B*27:05:02:40,B*27:05:02:41,B*27:05:02:42,B*27:05:02:43,B*27:05:03,B*27:05:04,B*27:05:05,B*27:05:06,B*27:05:07,B*27:05:08,B*27:05:10,B*27:05:11,B*27:05:12,B*27:05:13,B*27:05:14,B*27:05:15,B*27:05:17,B*27:05:18:01,B*27:05:18:02,B*27:05:19,B*27:05:20,B*27:05:21,B*27:05:22,B*27:05:23,B*27:05:25,B*27:05:26,B*27:05:27,B*27:05:28,B*27:05:29,B*27:05:30,B*27:05:31,B*27:05:33,B*27:05:34,B*27:05:37,B*27:05:39,B*27:05:40,B*27:05:41,B*27:05:42,B*27:05:43,B*27:05:44,B*27:05:45,B*27:05:46,B*27:05:47,B*27:05:48,B*27:05:52,B*27:05:53,B*27:05:54,B*27:05:55,B*27:05:56,B*27:05:57,B*27:05:58,B*27:05:59,B*27:05:60,B*27:05:61,B*27:05:62,B*27:05:63,B*27:08B*27:10,B*27:13:01,B*27:13:02,B*27:15,B*27:17,B*27:25,B*27:26,B*27:28,B*27:31,B*27:36,B*27:37,B*27:38,B*27:39,B*27:40,B*27:42,B*27:44,B*27:45,B*27:47,B*27:48,B*27:49,B*27:50:01,B*27:50:02,B*27:51,B*27:52,B*27:53,B*27:54,B*27:55,B*27:56,B*27:57,B*27:58,B*27:59N,B*27:60,B*27:61,B*27:62,B*27:63,B*27:64N,B*27:65N,B*27:66N,B*27:67,B*27:68,B*27:69,B*27:71,B*27:74,B*27:75,B*27:77,B*27:79,B*27:80,B*27:82,B*27:83,B*27:84,B*27:86,B*27:87,B*27:89,B*27:90:01,B*27:90:02,B*27:90:03,B*27:90:04,B*27:93,B*27:94N,B*27:95,B*27:96:01,B*27:96:02,B*27:97,B*27:98,B*27:99,B*27:100,B*27:101,B*27:103,B*27:104,B*27:105,B*27:108,B*27:110,B*27:111,B*27:112,B*27:113,B*27:114,B*27:115,B*27:116,B*27:117,B*27:118,B*27:120,B*27:121,B*27:122,B*27:123,B*27:124,B*27:126,B*27:128,B*27:129,B*27:131,B*27:132,B*27:133,B*27:134,B*27:135,B*27:137,B*27:139,B*27:140,B*27:142,B*27:143,B*27:144:01,B*27:144:02,B*27:145,B*27:146,B*27:147,B*27:148,B*27:149,B*27:152,B*27:155,B*27:156,B*27:158,B*27:159,B*27:160,B*27:161,B*27:162,B*27:163,B*27:165,B*27:166,B*27:167,B*27:169,B*27:170,B*27:171,B*27:172,B*27:173,B*27:174,B*27:175,B*27:176N,B*27:177,B*27:178:01,B*27:178:02,B*27:179,B*27:181,B*27:183,B*27:184,B*27:185Q,B*27:188,B*27:189,B*27:190,B*27:191,B*27:193,B*27:194,B*27:195,B*27:196,B*27:197,B*27:198,B*27:199,B*27:200,B*27:201,B*27:202,B*27:203,B*27:204:01:01,B*27:204:01:02,B*27:205,B*27:206,B*27:209,B*27:211,B*27:212N,B*27:213,B*27:214,B*27:216,B*27:217,B*27:220,B*27:221,B*27:223N,B*27:224,B*27:225N,B*27:226,B*27:228,B*27:229,B*27:230,B*27:231,B*27:232,B*27:234,B*27:235,B*27:236,B*27:237,B*27:239,B*27:240,B*27:241,B*27:243N,B*27:244,B*27:245,B*27:247,B*27:248,B*27:249,B*27:250,B*27:252,B*27:253Q,B*27:254N,B*27:255,B*27:257,B*27:258,B*27:259,B*27:260,B*27:261N,B*27:263N,B*27:264,B*27:265,B*27:266,B*27:267,B*27:268,B*27:270,B*27:271,B*27:272,B*27:273,B*27:274,B*27:275,B*27:276,B*27:277,B*27:278,B*27:281,B*27:282,B*27:283

Required Equipment and Materials

- Real-Time PCR Device
 - Compatible Devices*
 - Roche LightCycler 480
 - Roche LightCycler 96
 - Corbett Realtime PCR
 - BMS Mic
 - Biorad CFX Opus
 - Biorad CFX96
 - Slan Realtime PCR
 - Molarray MA6000
 - Himedia Insta Q96
 - Himedia Insta Q48 ve Insta Q48 M
- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

* Please contact for other compatible devices

Storage Conditions

- All components of the kit should be stored at -20°C

Channel / Parameter	Positive Control	Negative Control	NTC
FAM / HLA-B27	+	-	-
HEX / Internal Control	+	+	-

until the expiration date.

- Kit components should undergo freeze-thaw cycles a maximum of 3 times. If more than 3 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.
- Negative Control included in the kit should be stored at 2-8 °C after the initial thawing.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

- Preparation of 3 tubes is required for each sample. Reaction volumes are specified in the table. The same tubes should be prepared for the NTC.

Component	Positive Control Reaction	Negative Control Reaction	Sample Reaction
PCR Master Mix	18 µl	18 µl	18 µl
Positive Control	2 µl	-	-
Negative Control	-	2 µl	-
Sample	-	-	2 µl
Total Volume	20µl	20µl	20µl

Real – Time PCR Protocol

Cycle	Temperature (°C)	Time (Seconds)	Fluorescent Dye
1	95	600	
40	95	15	
	65	90	FAM,HEX

Data Analysis – Interpretation of Results

Positive Control (FAM Channel):

Validity Criteria: The positive control should show a clear amplification curve with a Ct (cycle threshold) value below the set threshold (typically $Ct \leq 36$). The curve should exhibit a smooth increase during the logarithmic phase.

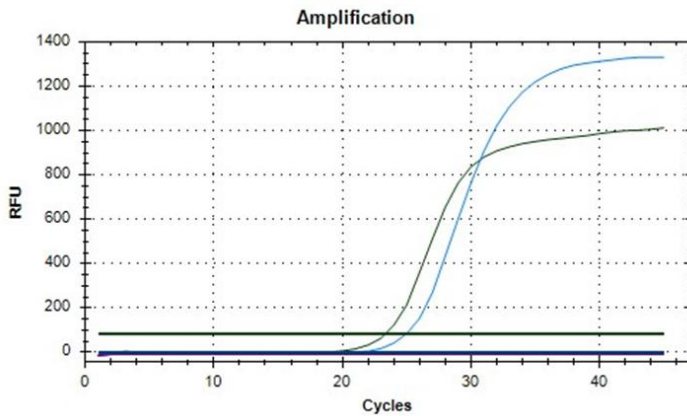
Result Evaluation: A clear amplification signal and an appropriate Ct value (e.g., $Ct < 36$) in the FAM channel indicate that the PCR reaction is functioning correctly and that the target DNA is being amplified. If the Ct value is high (e.g., $Ct \geq 36$) or the curve is flat, the positive control may be considered invalid.

Internal Control (HEX Channel):

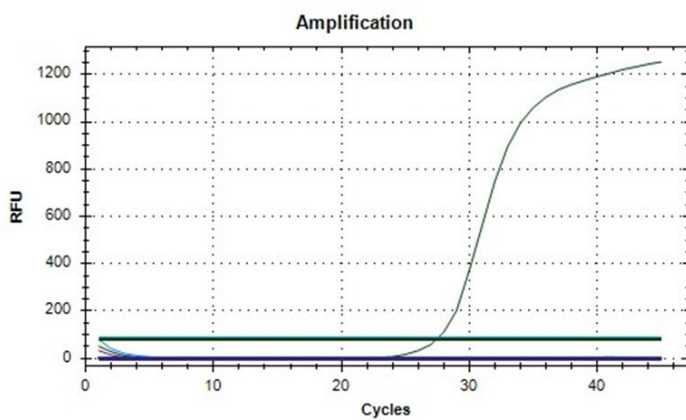
Validity Criteria: The internal control should show a clear amplification curve with a Ct value within an acceptable range (e.g., $Ct < 36$). The HEX channel should consistently exhibit a rising amplification curve in every run.

Result Evaluation: Successful amplification of the internal control in the HEX channel indicates that there are no inhibitors in the reaction and that the reaction conditions are appropriate. The internal control's curve must rise in each run to confirm consistent performance. If the HEX channel fails to show a rising curve or if the Ct value is outside the acceptable range, it suggests potential issues with the reaction and warrants further investigation.

Sample Images



HLA-B27 Positive Result (FAM+, HEX+)



HLA-B27 Negative Result (FAM-, HEX+)

Troubleshooting

Below are common issues that may arise during amplification-based assays and recommended corrective actions. Octobio kits are manufactured under strict quality controls—please verify each procedural step before suspecting kit integrity.

1. No Peaks Observed

Possible Causes & Recommended Actions

1. Inadequate Mixing

- *Cause:* Reaction components not fully homogenized.
- *Solution:* Vortex each master mix thoroughly before dispensing to ensure uniform reagent distribution.

2. Incorrect Annealing Temperature

- *Cause:* Thermal cycler set above the protocol's specified annealing temperature.
- *Solution:* Confirm the annealing temperature matches the kit protocol; do not deviate.

3. Poor DNA Quality or Quantity

- *Cause:* Suboptimal DNA isolation yields low-quality or insufficient template.
- *Solution:* Re-isolate DNA using a validated extraction method and verify concentration/purity (e.g. A_{260}/A_{280}).

4. Expired Reagents

- *Cause:* Kit or individual reagents past their expiration date.
- *Solution:* Discard any out-of-date components and use fresh Octobio reagents.

5. Pipetting Error

- *Cause:* Incorrect reagent volumes due to pipette miscalibration or operator error.
- *Solution:* Calibrate pipettes regularly and use low-retention tips; double-check pipetting steps.

2. Decrease in Fluorescence Intensity

Possible Causes & Recommended Actions

1. Light and Temperature Exposure

- *Cause:* Fluorescent probes degraded by prolonged exposure to ambient light or heat.
- *Solution:* Store reaction mixes and probes protected from light at recommended temperatures; minimize bench-top exposure.

2. Expired Reagents

- *Cause:* Fluorophore stability compromised in expired components.
- *Solution:* Replace with unexpired Octobio reagents.

3. Instrument Calibration

- *Cause:* Real-time PCR instrument optics not calibrated.
- *Solution:* Perform routine instrument maintenance and calibration according to the manufacturer's instructions.

3. Peak Observed Out of Expected Range

Possible Causes & Recommended Actions

1. Protocol Deviation

- *Cause:* Cycling parameters (e.g. ramp rate, hold times) not set as specified.
- *Solution:* Review and follow the exact thermal profile in the kit manual; repeat the run.

2. Contamination

- *Cause:* Carry-over or environmental contaminants leading to nonspecific amplification.

- **Solution:** Set up reactions in a clean area with filtered tips; include no-template controls; repeat under sterile conditions.

3. Plate Sealing or Well Leakage (Additional)

- **Cause:** Improper sealing allows evaporation or cross-well contamination.
- **Solution:** Use recommended optical seals and verify uniform well sealing before amplification.

Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• **Do not use expired kit.** • **Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test.** • **Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly.** • **Prepare the reaction mixture quickly on ice or a cold block.** • **Establish two separate work areas, one for isolation and the other for PCR.** • **Avoid transferring pipettes, tubes, and other working materials between the work areas.** • **Always use filtered sterile pipette tips.** • **Use separate lab coats and gloves for each work area.**

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**



:Number of Reactions



:Expiration Date



:Lot Number



:Reference Number



:Temperature Limit



:Refer to the User Manual



:Manufacturer



:Keep Away From Direct Sunlight

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