

# HELINI

## HLA-B\*27

### Real-time PCR Kit

[HLA-B\*27:01 to B\*27:73]

Instructions for use

**For use with:** Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI], Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

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HELINI Biomolecules, Chennai, INDIA

### Intended Use

The HELINI HLA B27 Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of HLA-B27 Genotype [HLA-B 27:01 to 27:73] in human genomic DNA extracted from EDTA whole blood.

### Kit components

| Components                                      | Volume Per reaction | Number of vials | Volume Per vials |
|---|---------------------|-----------------|------------------|
| Probe PCR Master Mix                            | 10µl                | 1               | 250µl            |
| HLA B27 Primer Probe Mix [HLA B27 PP mix]       | 2.5µl               | 1               | 65µl             |
| Endogenous Primer Probe Mix [Endogenous PP Mix] | 2.5µl               | 1               | 65µl             |
| HLA B27 Positive control                        | 10µl                | 1               | 150µl            |
| Water, PCR grade                                |                     | 1               | 4ml              |

### Storage

- The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

### Material and instruments required

- Real-time PCR instrument having FAM & HEX channels
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

*[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer's instructions and recommendations.]*

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### Product Use Limitations

- All reagents may exclusively be used in molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

### Technical Assistance

For technical assistance and more information, please contact;  
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## Product description

HELINI HLA B27 mutation Real-time PCR Kit constitutes a ready-to-use system for the detection of the HLA-B27 genotype using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification for the direct detection of the specific amplicon in fluorescence channels FAM. In addition, it contains an endogenous control amplification system amplifies human gene detected in HEX channel which identify possible PCR inhibition and DNA purification. External mutation positive control is supplied to assist the run.

## Specificity

HLA-B27 primer and probe have been designed for the specific and exclusive *in vitro* detection of HLA-B27 genotypes. The Primer Probe can able to detect all the subtypes ranging from **B27:01 to B27:73**. The target sequence is highly conserved and has previously been shown to be a good genetic marker for the detection of HLA-B27 genotyping. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

## Dynamic linear range

The linear range was evaluated by analyzing a logarithmic dilution series of DNA concentrations ranging from 100ng/μl to 10ng/μl. At least six replicates per dilution were analyzed. The slopes are in expected limit in the recommended DNA concentration of 10ng/μl.

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## Analytical Sensitivity

The analytical sensitivity is defined as the concentration of DNA molecules (ng/μl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified HLA B27 specific DNA from 1ng/μl to 100ng/μl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 2ng/μl

## Note:

### DNA Purification

Strongly recommended to use minimum 300μl of EDTA whole human blood for the DNA purification.

Purified DNA is the starting material for the Real-time PCR assay. The quality of the purified DNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for DNA purification is compatible with real-time PCR technology.

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 3min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

### Endogenous control

Human gene is given as endogenous control. It amplifies a single copy human gene from the test samples. A successful amplification indicates that test samples are properly collected and has its biological property.

The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the endogenous control template does not interfere with detection of the mutation even when present at low copy number. The endogenous control is detected through the HEX channel and gives a CT value of 25 +/-7.

### Detection Protocol

#### Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

| Components            | Volume per reaction |
|-----------------------|---------------------|
| Probe PCR Master Mix  | 10µl                |
| HLA B27 PP Mix        | 2.5µl               |
| Endogenous PP Mix     | 2.5µl               |
|                       | <b>15µl</b>         |
| <b>Purified DNA</b>   | <b>10µl</b>         |
| Final reaction volume | 25µl                |

**Negative Control setup [NTC]**

Add 10µl of PCR grade water.

**Positive Control setup**

Add 10µl of the Positive control

Centrifuge PCR vials briefly before placing into thermal cycler.

*[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

**Programming Thermal cycler**

|                          |           |
|--------------------------|-----------|
| <b>Sample volume</b>     | 25µl      |
| <b>Fluorescence Dyes</b> | FAM & HEX |
| <b>Passive reference</b> | None      |
| <b>Ramping rate</b>      | Default   |

**Thermal Profile**

|                      | <b>Step</b>                  | <b>Time</b> | <b>Temp</b> |
|----------------------|------------------------------|-------------|-------------|
|                      | Taq enzyme activation / Hold | 15min       | 95°C        |
| <b>40<br/>cycles</b> | Denaturation                 | 20sec       | 95°C        |
|                      | Annealing/Data collection*   | 20sec       | 56°C        |
|                      | Extension                    | 20sec       | 72°C        |

| <b>Data collection/Acquisition</b> | <b>Targets</b>     |
|------------------------------------|--------------------|
| FAM                                | HLA B27            |
| HEX                                | Endogenous control |

**Reading the graph:****Step-1 –Endogenous control Validation**

Select the test samples alone for the endogenous control analysis. Select HEX dye and view the graph of endogenous control amplification. A successful amplification Ct value must be within Ct 25 +/- 7.

This range indicates NO PCR inhibition in the reaction. Any sample value goes beyond Ct 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Internal control will not get amplified in the negative and positive controls. Ignore a late noise HEX amplification graph in the NTC and Positive control wells.

**Step-2 – FAM - Negative and Positive control validation**

Select the NTC and Positive control, select FAM channel, and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The Positive control must be amplified.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

**Step-3 –FAM - Test Sample status**

In FAM channel, select test sample well one by one, analyze the graph/amplification.

**Qualitative interpretation of results:**

| Test Sample | Negative control | Positive control | Endogenous Control | Interpretation                         |
|-------------|------------------|------------------|--------------------|--|
| Positive    | Negative         | Positive         | Positive           | HLA B27 genotype specific DNA detected |
| Negative    | Negative         | Positive         | Positive           | Not detected                           |
| Negative    | Negative         | Negative         | Negative           | Experiment fail                        |
| Positive    | Positive         | Positive         | Positive           | Experiment fail                        |

### Limitations

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.






Potential mutations within the target regions of the genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the HELINI HLA B27 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

### Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI HLA B27 Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

### Explanations of symbols

|   |                                    |
|---|------------------------------------|
|    | In vitro diagnostic medical device |
|    |                                    |
|    | Catalogue number                   |
|   | Pack size – number of tests        |
|  | Manufacturer                       |



Manufactured by

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