# HLA-B27 RT-PCR Detection Kit for Exon 2 & Exon 3

Σ 100 Reactions | -20°C

## INTENDED USE

HLA-B27 RT-PCR detection kit for Exon 2/Exon 3 is a real-time PCR test kit for the detection of Human Leukocyte Antigen B27 in whole blood. HLA-B27 is strongly associated with Ankylosing Spondylitis (AS) and other related inflammatory diseases, such as Psoriasis, Inflammatory Bowel disease and Reactive Arthritis. Testing for HLA-B27 can aid in early diagnosis and management of these conditions.

## BACKGROUND

Human Leukocyte Antigen (HLA), found on White Blood Cells, are proteins that help the body's immune system tell the difference between its own cells, foreign and harmful substances. Although most HLAs protect your body from harm, HLA-B27 is a specific type of protein that contributes to immune system dysfunction. The presence of HLA-B27 in white blood cells can cause your immune system to attack those otherwise healthy cells. When this occurs, it can result in an autoimmune disease or immune-mediated disease, such as juvenile rheumatoid arthritis or ankylosing spondylitis (AS). AS is an inflammatory disease that, over time can cause some of the bones in the spine to fuse. This fusion makes the spine less flexible and can result in a hunched posture.

## PRODUCT DESCRIPTION

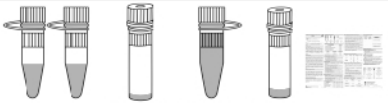
HLA-B27 RT-PCR Detection Kit for Exon 2/Exon 3 is an *in-vitro* RT-PCR qualitative assay for the specific detection of Human Leukocyte Antigen B27 in whole blood. HLA-B27 RT-PCR Detection Kit for Exon 2 and Exon 3 is a multiplex assay kit where detection of (Exon 2 /Exon 3) and 1 internal control (cyp17a1) in a single tube reaction. This kit is intended for "Professional Use only".

## ANALYTICAL SPECIFICATION

The specificity of the kit is 100% with 100% sensitivity. The limit of detection of the kit for Exon 2/3 target gene is 10 copies per µL.



## MATERIALS PROVIDED



| 2X Master Mix (MM) 2 * 625 µL | 20X Primer & Probe Mix (PPMx) 125 µL | Nuclease Free Water (NFW) 1000 µL | Positive Test Control (PTC) 100 µL | Instruction for use (1 No) |
|---|---|---|---|---|

## MATERIALS PROVIDED

| KIT COMPONENTS | VOLUME |
|---|---|
| 2X Master Mix (MM) | 2*625 µL |
| 20X Primer and Probe Mix (PPMx) | 125 µL |
| Nuclease Free Water (NFW) | 1000 µL |
| Positive Test Control (PTC) | 100 µL |

| TARGET | REPORTER | QUENCHER |
|---|---|---|
| Exon 2/3 | HEX/VIC | BHQ2 |
| Internal control cyp17a1 | TEXAS RED/ROX | BHQ2 |

## STORAGE AND HANDLING

- Store all HLA-B27 kit components at -20 °C.
- Do not repeatedly freeze-thaw reagents as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C. Recommended Freeze thaw cycle is 5 times.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacturing.

## PRECAUTIONS

- This product is recommended to be used by the trained professional under laboratory Condition.
- Treat all the specimen/sample as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store all the collected human samples away from the kit components before use to avoid contamination.
- Handle master mix and template preparation separately and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.
- Do not open the reaction tubes/plates post-amplification, to avoid contamination with amplicons.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Do not use kit components that have passed their expiration date.

## WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation and expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction
- Dispose the PCR plates with patient samples "sealed" post run to avoid potential infection to the operators and contamination of the lab.

## REACTION MIXTURE - 25 µL

| Reagents | 1 Rxn | 20 Rxn | 50 Rxn | 100 Rxn |
|---|---|---|---|---|
| 2X Master Mix | 12.5 µL | 250 µL | 625 µL | 1250 µL |
| 20X Primer & Probe mix | 1.25 µL | 25 µL | 62.5 µL | 125 µL |
| Nuclease Free Water | 5.25 µL | 105 µL | 262.5 µL | 535 µL |
| Total | 19 µL | 380 µL | 950 µL | 1900 µL |

*Add 6 µL of the test DNA per reaction*



## TEST PREPARATION/REACTION SET-UP

1. Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
2. Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors. (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1)
3. Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
4. Spin down the tubes and dispense 19 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 6 µL of nuclease free water in NTC wells.
5. Carefully add 6 µL samples kept on ice in the designated wells in template addition room. Add 2 µL of PTC in a separate hood and make up the volume by adding 4 uL of nuclease free water. The assay should be run along with positive controls and negative controls.

## THERMAL CYCLIC CONDITIONS

| STEP | TEMP °C | TIME | DETECTION | CYCLE |
|---|---|---|---|---|
| Hold | 95 | 2 Min | Off | 1 |
| PCR and Detection | 95 | 15 Sec | Off | 40 |
| | 58 | 30 Sec | On | |

## READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC reactions , it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected Ct value for each target included in the test.
- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.

## RESULTS INTERPRETATION

| For Exon 2/Exon 3 | cyp17a1 Internal Control | Assay result |
|---|---|---|
| Ct < 40 | Ct ≤ 40 | Positive |
| Ct = Undetermined or Ct ≥ 40 | Ct ≤ 40 | Negative |
| Ct = Undetermined or Ct ≥ 40 | Ct ≥ 40 Undetermined | Invalid. Re-purify the nucleic acid from the sample, then repeat the test. |



## DATA INTERPRETATION

| Exon 2/Exon 3 HEX/VIC | IC Texas Red/ROX | RESULTS INTERPRETATION |
|---|---|---|
| + | + | Exon 2/ Exon 3 detected |
| - | + | Exon 2/ Exon 3 not detected |
| - | - | Invalid results repeat the DNA extraction and re-run |

## TROUBLESHOOTING

**Positive control showed no amplification**

*Inappropriate storage of reagents*

- Store the reagents at recommended temperature for their optimal performance.
- Avoid freeze-thaw more than the recommended number of times
- Check the expiry of reagents.

**Negative controls are positive**

*Causes - Cross-contamination*

- Follow good laboratory practices to avoid contamination issues.
- Use a new batch of reagents and repeat the experiment.

**Abnormal plot and/or low ΔRn values in amplification curve**

*The baseline was set improperly (some samples have CT values lower than the baseline value)*

- Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold)

**An amplification signal is detected in the early cycles**

*Dilute the sample to increase the CT value*