Quantiplus® HLA-B27 Detection Kit (Real-Time Qualitative PCR Kit)

QL-HLA-25 : 25 rxns
QL-HLA-50 : 50 rxns
QL-HLA-100 : 100 rxns

IVD

PI/QL-HLA-02

Intended Use

Quantiplus® HLA-B27 Detection Kit is an in vitro Real-Time PCR Kit for the qualitative detection of HLA B27 alleles (subtypes B*2701-27174) in human blood. The kit contains Amplification Mix with specific Primers and Probes and Positive Control.

Background Information

HLA-B27 is strongly correlated with Ankylosing Spondylitis, 90% of the patients with Rheumatic disease have HLA-B27 alleles compared with 10% of healthy controls. Other diseases associated with HLA-B27 are Reiter's syndrome, Acute anterior uveitis, Reactive arthritis and bowel infections with *Yersinia, Salmonella* and *Shigella*.

Kit components

Color Coding	Contents	Description	25 rxns (QL-HLA-25)	50 rxns (QL-HLA-50)	100 rxns (QL-HLA-100)
Amber	Huwel HLA Ready Mix	HLA and Internal Control Probes and Primers with Amplification Mix	1 x 375 µL	1 x 750 µL	2 x 750 µL
Red	Huwel HLA PC	HLA Positive Control	1 x 100 µL	1 x 100 µL	2 x 100 µL
White	Huwel PW	Purified Water	1 x 500 µL	1 x 500 µL	1 x 1 mL

Note: Please pay attention to the cap color coding and the tube contents

Huwel PW: Molecular Biology Grade Purified Water

Storage and Transportation Conditions

The kit should be transported at temperature below −20 °C. The kit is stable until the expiry date printed on the package, if the storage temperature is within −20 ±5 °C. More than 4X freezing and thawing cycles reduces the assay sensitivity. For intermittent usage the reagents should be frozen in aliquots.

Technical Specification

Target Sequence	Specific region in Exon 2 and 3
Specificity	100%
Limit of Detection	0.4 ng/µL
Validated Specimen	K2EDTA-Blood

Assay Procedure

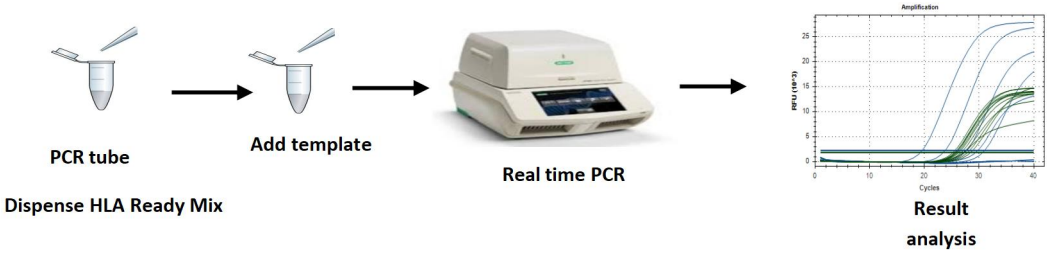
DNA Extraction

Quantiplus® HLA-B27 Detection Kit has been validated using the following Nucleic acid extraction kits:
Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Genomic DNA Extraction Kit (Cat. No. HL-GDX-100)	1mL	100 µL
2.	Huwel Nucleic Acid Extraction Kit-version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL
3.	QIAamp®Blood DNA Mini Kit (Cat. No. 51104)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other Nucleic acid extraction Kits.

qPCR Protocol Flow



Preparation of Reaction Master mix

Components	Volume per reaction (For 25µL)
Huwel HLA Ready Mix	15.0
Extracted DNA/ Huwel HLA PC/Huwel PW	10.0

Note: IC is endogenous
It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes and centrifuge briefly before proceeding to thermal cycler.

Cycling Conditions

Steps	Temperature (°C)	Time
1 (Initial Denaturation)	95	15 min.
40 (PCR cycling)	95	15 sec.
	60*	1 min.
*Plate read/Data acquisition in FAM and Yakima Yellow / HEX/VIC channel		

Result and Interpretation

Interpret the values for unknown samples based on the observations described in the following table. No amplification should be observed in the NTC. The Ct values of ≤36 Ct for HLA-B27 DNA and ≤30 Ct for endogenous internal control of unknown samples should be considered for positive sample interpretation.

S.No	FAM (HLA-B27)	YAKIMA YELLOW/ HEX/ VIC (Endogenous Control)	Fluorophore ← / Interpretation ↓	Conclusion
1	√	√	HLA-B27 DNA detected	Proceed for further analysis
2	√	-		
3	-	√	HLA-B27 DNA Not detected	
4	-	-	Possible inhibition of PCR	Dilute the DNA sample(1:10) and repeat the Assay

Note: All the Target channels (FAM, and Yakima Yellow /VIC/HEX) to be analyzed individually.

Validated Instruments

- Thermo QS5 Real-Time PCR System
- Bio-Rad ™ CFX 96

HLSS Manufacturing Pvt Ltd
Plot No's M14,M15, M16, TSIIC Medical device park
Sultanpur villiage, Ameenpur Mandal,
SangareddyDist, TS-502319