Factor V Leiden Real-Time PCR Detection kit

100 Reactions | -20°C

INTENDED USE

Factor V Leiden Real-Time PCR Detection kit is designed to detect G1691A mutation in DNA Extracted from the whole blood for human factor V gene by real-time Polymerase Chain Reaction (Real-Time PCR) method. The method is based on the amplification and detection of the target sequence using allele-specific fluorophore-labeled probes.

SAMPLE TYPE

Human whole blood

BACKGROUND

The coagulation factor V, a large 330-kD plasma glycoprotein, is encoded by the F5 gene. Factor V that circulates with less or no activity. Factor V is converted to the active form, factor Va, by thrombin (Factor II). Activated factor V serves as an essential protein in the coagulation pathway and acts as a cofactor for the conversion of prothrombin to thrombin by factor Xa . Factor Va is inactivated by activated protein C. Parahemophilia, also known as Factor V Deficiency, is caused due to homozygous or compound heterozygous mutations in the F5 gene. Factor V deficiency is a rare autosomal recessive bleeding disorder with phenotypic variations. A heterozygous 1691G-A transition in exon 10 of the F5 gene, resulting in an arg506-to-gln (R506Q) substitution was identified by Bertin et al (1994). The presence of R506Q mutation to prevention of inactivation of activated factor V, leading to thrombosis.(OMIM,* 612309 COAGULATION FACTOR V; F5).The Factor V Leiden mutation (c.G1691A) is a crucial predisposing factor for the occurrence of thrombosis and molecular screening of factor V mutation is essential to assess the risk of thrombosis in asymptomatic patients with family history of thromboembolic episode.

PRODUCT DESCRIPTION

Factor V Leiden Real-Time PCR Detection kit is designed to detect G1691A mutation in the gene for human factor V Leiden by the real-time Polymerase Chain Reaction (Real-Time PCR) method. The method is based on the amplification and detection of the target sequence using allele-specific fluorophore-labeled probes. The target sequence is a single nucleotide guanine/adenine polymorphism in site 1691 (G1691A). The presence of the wild-type allele (G1691G) is detected in the HEX fluorescent channel and the mutant allele (A1691A) in the FAM fluorescent channel. In case of the heterozygous genotype (G1691A) a signal is detected in both channels.

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
Master Mix	2x625 µL
Primer and Probe mix	125 µL
Nuclease Free Water	1000 µL
Positive Test Control (PTC)	100 µL
Instruction for use	1 No.

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables
PCR Plates/tubes
PCR plate covers/tube caps

COMPATIBLE INSTRUMENTS

Real-Time PCR, instruments like, BIORAD-CFX96, THERMO -QS5, QIAGEN-ROTOR - GENE Q and other instruments which supports HEX (535 nm - 556 nm) and FAM (495 nm - 520 nm)

TARGET	REPORTER	QUENCHER
Factor V Wild Type	HEX	BHQ2
Factor V Mutant	FAM	BHQ1

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

- Store all Factor V Leiden Real-Time PCR Detection Kit reagents at -20°C.
- Do not repeatedly freeze-thaw reagents more than five times as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C.
- 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

- It is recommended that this product is used by personnel specially instructed and trained in real-time PCR and in-vitro diagnostics procedures.
- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate areas for master mix and template preparation 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.

REACTION MIXTURE - 25 µL

Reagents	1 Rxn	20 Rxn	50 Rxn	100 Rxn
Master Mix	12.5 µL	250 µL	625 µL	1250 µL
Primer & Probe mix	1.25 µL	25 µL	62.5 µL	125 µL
Nuclease Free Water	3.25 µL	65 µL	162.5 µL	325 µL
Total	17 µL	340 µL	850 µL	1700 µL

Add 8 µL of the test DNA per reaction

SAMPLE PREPARATION

DNA should be extracted from freshly taken Human whole blood using any approved DNA extraction methods. Store the extracted DNA samples at - 20 ℃ for further use.

TEST PREPARATION/REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
- 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).
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
- Spin down the tubes and dispense 17 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 8 µL of nuclease free water in NTC wells.
- Carefully add 8 µL of DNA 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 6 µL of nuclease free water. The assay should be run along with positive controls and negative controls.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLIC CONDITIONS

STEP	TEMP °C	TIME	DETECTION	CYCLE
Hold	95	1 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.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings.
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.
- Negative results do not exclude possibility of infection and should not be used as the sole basis for the treatment.

RESULTS INTERPRETATION

For all target genes - Wild Type	For Mutant	Assay result
Ct < 37	Ct ≤ 37	Heterozygous condition of mutation
Ct = Undetermined or Ct ≥ 37	Ct ≤ 37	Homozygous condition of mutation
Ct < 37	Ct = Undetermined or Ct ≥ 37	Homozygous condition of wild type
Ct = Undetermined or Ct ≥ 37	Ct ≥ 37 Undetermined	Invalid. Re-purify the nucleic acid and repeat the test.

DATA INTERPRETATION

Wild Gene	Mutant Gene	Results Interpretation
+	-	Wild- Type gene in Homozygous
-	+	Mutation in Homozygous condition
+	+	Mutation in Heterozygous condition

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation & 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.

TROUBLESHOOTING

Positive control showed no amplification

Inappropriate storage of reagents

- Store the reagents at recommended temperature for their optimal performance.
- Avoid repetitive freezing and thawing.
- 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