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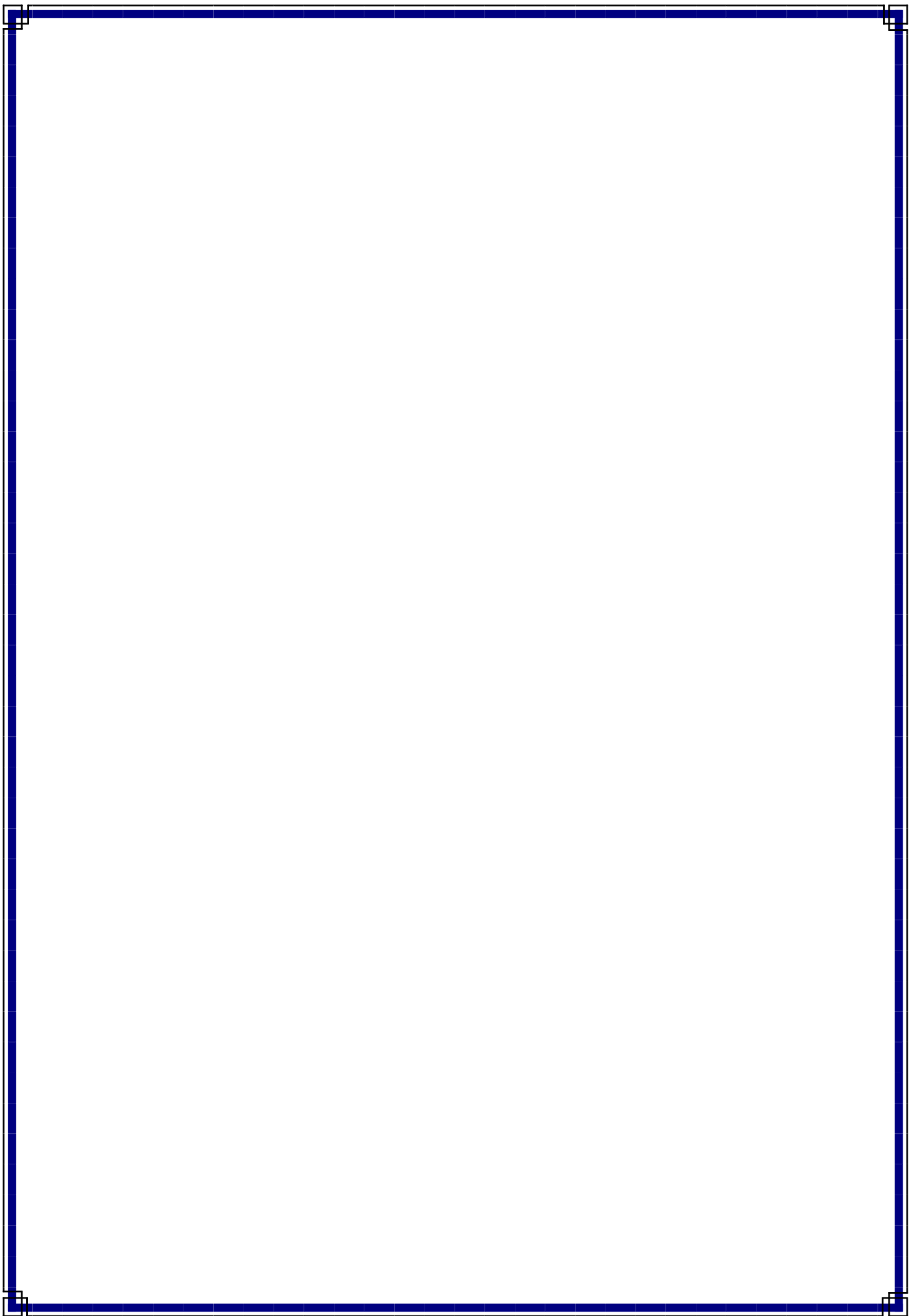
FVL Genotyping Kit

User Manual

For in vitro Diagnostic Use

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SimReal Factor V Leiden Genotyping Kit

Product Description:

SimReal Factor V Leiden Genotyping Kit is designed to determine the genotype of factor V Leiden gene, G1691A mutation, related to thrombophilia using Real-time PCR technology. Mutation detection is based on amplification and detection of distinct alleles using corresponding labeled probes. The probes targeting normal (G1691) and mutant (1691 A) alleles are labeled with FAM and HEX flouochrome, respectively.

Contents of the kit

Reagents	Labels	volume
Master mix 2X	2X Real Time Mix	500 ul
Primer and probe Mix	Oligomix F V Leiden	100 ul
Heterozygote control	HET F V Leiden Positive control	20 ul
Mutant homozygote for Factor V Leiden	HOMO MUT F V Leiden Positive control	20 ul
Wild type homozygote for Factor V Leiden	HOMO WT F V Leiden Positive control	20 ul
ddH2O		500 ul

Storage condition

- Each component of the kit should be stored at -20°C
- All test reagents are stable until their expiration date at recommended conditions.
- If the kit is to be used only occasionally, it is recommended to aliquot the reagents.
- Repeated freezing and thawing (> 3x) may reduce the sensitivity of the assay. It is recommended not to let the reagents undergo more than two freeze/thaw cycles.
- Primer and probe Mix contain fluorescent molecules: it is recommended to store away from any direct light source.

Notes before starting

- Read carefully and completely the instruction manual, before starting the kit procedure.
- Keep the product away from heating and light sources.
- Always start with the cycling condition and Oligomix concentrations specified in this protocol.
- Thaw all components thoroughly at room temperature before starting the assay. Mix the solutions well by inverting the tubes several times (do not vortex!), then centrifuge briefly.

- Prepare the reaction mix according to Table 1 on ice or on the cooling block.
- Use filter pipette tips
- Use disposable powder-free gloves and change your gloves frequently.

Table 1. Reaction Setup

component	Final concentration	Volume/reaction
Reaction Mix		
2x PCR Master Mix	1x	10ul
10 X oligomix	1x	2ul
ddH2O	-	7ul
Template DNA	≤100ng/reaction	1ul
Total reaction volume		20ul

- Add template DNA (≤100ng / reaction) to the individual PCR tubes or wells containing the reaction Mix.
- Program the Real Time PCR device according to Table 2.
- Place the PCR tubes or plates in the Real Time cycler, and start the cycling program.

Table 2 . Cycling conditions

Step	Time	Temperature	
PCR initial heat activation	2 min	95°C	1X
Denaturation	15 s	95°C	
Annealing/Extension*	60 s	60°C	35X

* Acquire florescent signal in green and yellow channels

Optional: Check the specificity of PCR product(s) by agarose gel electrophoresis.

Required Materials and Devices, not provided

- DNA extraction kit
- Appropriate PCR tubes or plate
- Disposable sterile filter-tips (range: 0,5-10 µL; 2-20 µL; 10-100 µL; 20-200 µL; 100-1000 µL)

Ordering information:

info@sim-biolab

Product name	Cat. No.	Technology	Package
SimReal Factor V G1691A Genotyping Kit	SBL11-360/50	Real-time PCR	50 reactions
SimReal Factor V G1691A Genotyping Kit	SBL11-360/100	Real-time PCR	100 reactions

Related products:

Product name	Cat. No.	Technology	Package
SimReal PAI-1 Genotyping	SBL11-68/50	Real-time PCR	50 reactions
SimReal PAI-1 Genotyping	SBL11-68/100	Real-time PCR	100 reactions
SimReal MTHFR C677T Genotyping Kit	SBL11-196/50	Real-time PCR	50 reactions
SimReal MTHFR C677T Genotyping Kit	SBL11-196/100	Real-time PCR	100 reactions
SimReal MTHFR A1298C Genotyping Kit	SBL11-798/50	Real-time PCR	50 reactions
SimReal MTHFR A1298C Genotyping Kit	SBL11-798/100	Real-time PCR	100 reactions
SimReal Prothrombin Genotyping Kit	SBL11-914/50	Real-time PCR	50 reactions
SimReal Prothrombin Genotyping Kit	SBL11-914/100	Real-time PCR	100 reactions