

## Warfarin dose VKORC1/CYP2C9 PCR Kit



### *in vitro* Diagnostics

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* medical diagnostic device and it is designed for professional use in specialized clinical laboratories.

### Kit Composition

Cat. No.	WARF/025 25 reactions	WARF/050 50 reactions
MasterMix Warfarin dose CYP2C9*2	1 x 450 µl	2 x 450 µl
MasterMix Warfarin dose CYP2C9*3	1 x 450 µl	2 x 450 µl
MasterMix Warfarin dose VKORC1	1 x 450 µl	2 x 450 µl
Positive Control Warfarin-wt	1 x 200 µl	1 x 200 µl
Positive Control Warfarin-mut	1 x 200 µl	1 x 200 µl
Positive Control Warfarin-het	1 x 200 µl	1 x 200 µl

### Storage and Transportation Conditions

Recommended storage temperature: -20 to -80 °C. If this optimum temperature range is maintained, the kit shall be stable for the period specified on the label. Repeated thawing and freezing of the kit may result in lower detection quality. Therefore the manufacturer recommends dividing the kit into smaller aliquot parts and maintain them from -20 to -80°C until the moment of their use. Positive Control can be maintained from 2 to 8 °C.

### Product Information

Warfarin is the most frequently used anticoagulative drug for patients suffering from thromboses in the blood stream. This drug features low therapeutic index (small difference between toxic and therapeutic doses) and significant individual variability in the drug dosage. This variability is caused by single-nucleotide polymorphisms in the CYP2C9 and VKORC1 genes. CYP2C9 and VKORC1 gene polymorphisms are related to lowered warfarin metabolism and the resulting need to supply the patient with a lower dose of the drug. The DNA polymorphism analysis makes it possible to determine a suitable warfarin dose according to the genetic characteristics of each individual person and prevent undesirable treatment effects.

## Method Principles

The PCR kit is designed for the detection of polymorphisms in the sub-unit 1 of the vitamin K epoxide reductase enzyme complex encoded by the VKORC1 (-1639G>A) gene and allelic variants of the CYP2C9\*2 (430C>T) and CYP2C9\*3 (1075A>C) genes encoding the P450 2C9 cytochrome using the real-time Polymerase Chain Reaction (PCR). These polymorphisms significantly impact the genetic component of the individual sensitivity variability to warfarin. The kit is designed for *in vitro* diagnostics.

The PCR kit is designed for the detection of polymorphism 1173C>T in VKORC1 gene. The -1639A and 1173T SNPs are in near complete linkage disequilibrium across populations. The -1639G>A and 1173C>T SNPs are similarly predictive of warfarin dose, and thus only one needs to be considered in warfarin dosing decisions.

Evaluation: standard homozygote (wild type), mutant homozygote, heterozygote.

## User Manual

### Sampling and sample storage

Sampling does not require any special preparation of the patient. Usually 3 ml of peripheral blood are sampled into a tube with EDTA (the same procedure as blood count sampling). The blood can be kept in a refrigerator at 2-8 °C for 3 days and then it should be frozen from -80 °C to -20 °C.

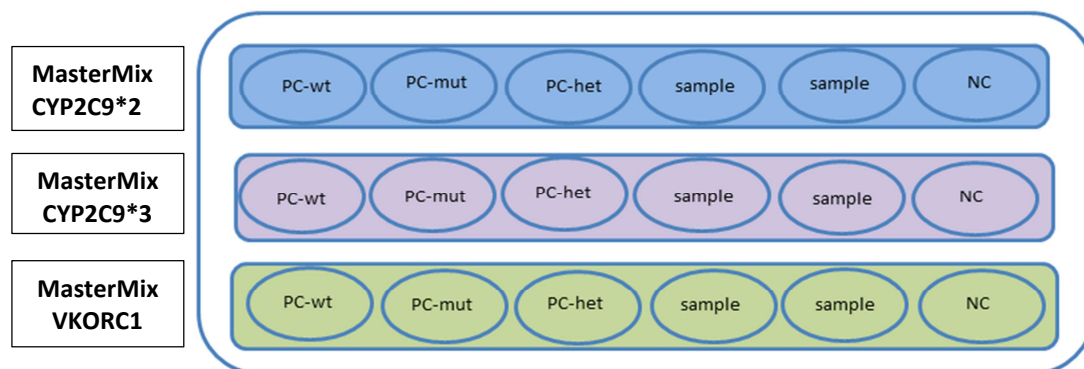
### DNA isolation

DNA isolation should be performed by isolation kits available at the market according to specific protocols. The manufacturer recommends to use the PathogenFree DNA Isolation Kit (GeneProof).

### PCR Reaction Preparation and Processing

1. Add 18 µl of the relevant MasterMix (MM CYP2C9\*2 or CYP2C9\*3 or VKORC1) into PCR tubes.

#### An example of sample laying while using the Warfarin dose VKORC/CYP2C9 PCR Kit

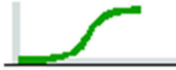


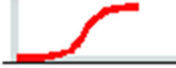









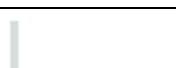








2. Add 2 µl of the DNA sample or 2 µl of Positive Control into the individual tubes. The final reaction mix volume will be 20 µl.
3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
- 4.

### Amplification Program

Number of cycles	Description	Temperature/Time	Reading of the fluorescence signal
1	initial denaturation	95 °C/10 min.	
	denaturation	95 °C/10 sec.	
40	annealing	64 °C/20 sec.	Reading of the fluorescence signal FAM and HEX
	extension	72 °C/20 sec.	

## Detection Qualitative Evaluation

	FAM	HEX	Result
MasterMix CYP2C9*2			C/C - standard homozygote ( <i>wild type</i> )
			T/T - mutant homozygote
			C/T - heterozygote
MasterMix CYP2C9*3			A/A - standard homozygote ( <i>wild type</i> )
			C/C - mutant homozygote
			A/C - heterozygote
MasterMix VKORC1			G/G - standard homozygote ( <i>wild type</i> )
			A/A - mutant homozygote
			G/A - heterozygote
			Invalid

## Technical specification

Technology	real - time PCR
Target sequence	DNA sequence of the CYP2C9 genes (P450 2C9 cytochrome) and VKORC1 (vitamin K epoxide-reductase)
Specificity	Single-nucleotide polymorphism CYP2C9*2 (C/T),CYP2C9*3 (A/C),VKORC1 (C/T)
Qualitative demonstration	present x not present
Evaluation	CYP2C9*2- <b>C/C</b> - standard homozygote (wild type); <b>T/T</b> -mutant homozygote; <b>C/T</b> - heterozygote CYP2C9*3- <b>A/A</b> - standard homozygote (wild type); <b>C/C</b> - mutant homozygote; <b>A/C</b> - heterozygote VKORC1- <b>G/G</b> - standard homozygote (wild type); <b>A/A</b> - mutant homozygote; <b>G/A</b> - heterozygote
Sample types	Whole blood in EDTA
Storage	-80 °C to -20 °C
Quality Control	Regularly tested by the <b>INSTAND e.V.</b> External Quality Assessment Panels - for results see <a href="http://www.geneproof.com">www.geneproof.com</a>
Certification	CE IVD for <i>in vitro</i> diagnostics

## Supported real-time PCR devices

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. GeneProof Warfarin dose VKORC/CYP2C9 PCR Kit has been validated for the following devices:

- **Rotor-Gene™ 3000/6000/Q**
- **LightCycler® 2.0/480**
- **SLAN ®Real-time PCR System**
- **CFX 96 BioRad**

For detailed information about the PCR kit use with specific devices see the Manufacturer's web site ([www.geneproof.com](http://www.geneproof.com)) or request the information from your kit supplier. If you want to use the kit with other real-time devices, contact the manufacturer: [support@geneproof.com](mailto:support@geneproof.com)

## Warning

The only valid Package Insert for the particular kit is the package insert included in the kit or it is available for the particular lot from manufacturer.

Be very careful when handling the Positive Control or the clinical material – incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

The kit should be disposed of after use according to the current legal regulations considering the fact that the kit doesn't contain any dangerous, infectious or toxic components that would be subject to special safety regulations and the packaging materials are made of paper and polypropylene.

If you have any questions please contact the Product Specialized Support Department.

### **Customer service and technical support**

Phone: +420 543 211 679

Fax: +420 516 770 824

E-mail: [support@geneproof.com](mailto:support@geneproof.com)

### **Mail orders to:**

Phone: +420 543 211 679

Fax: +420 516 770 824

E-mail: [sales@geneproof.com](mailto:sales@geneproof.com)