GeneProof
Molecular diagnostics for your routine

Package insert

GeneProof VRE PCR Kit

CE IVD

In vitro diagnostic medical device

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

KIT CONTENT

	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid isolation and PCR inhibition control		
REF	**VRE/ISIN/025** 25 rxn	**VRE/ISIN/050** 50 rxn	**VRE/ISIN/100** 100 rxn	**VRE/ISEX/025** 25 rxn	**VRE/ISEX/050** 50 rxn	**VRE/ISEX/100** 100 rxn
MasterMix VRE	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
Positive Control VRE	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
Internal Standard VRE	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

Target Sequence	*vanA* and *vanB* genes
Specificity	vancomycin-resistant Enterococci (*Enterococcus faecalis* a *Enterococcus faecium*)
Sensitivity (LOD)	for *vanA* reaches 1,398 copy/µl with 95 % probability for *vanB* reaches 1,026 copy/µl with 95 % probability
Sample Type	whole blood, sputum, stool, urine
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

METHOD PRINCIPLES

The PCR kit is designed for the detection of Vancomycin-Resistant Enterococci (VRE), *Enterococcus faecalis* and *Enterococcus faecium*, by the real-time Polymerase Chain Reaction (PCR) method. The VRE detection is based on the amplification of a specific conservative DNA sequence in the area of the *vanA* and *vanB* genes and measuring the amplification product concentration growth using PCR process and fluorophore labelled probes. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction (ISIN version) and possibly also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit utilizes the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

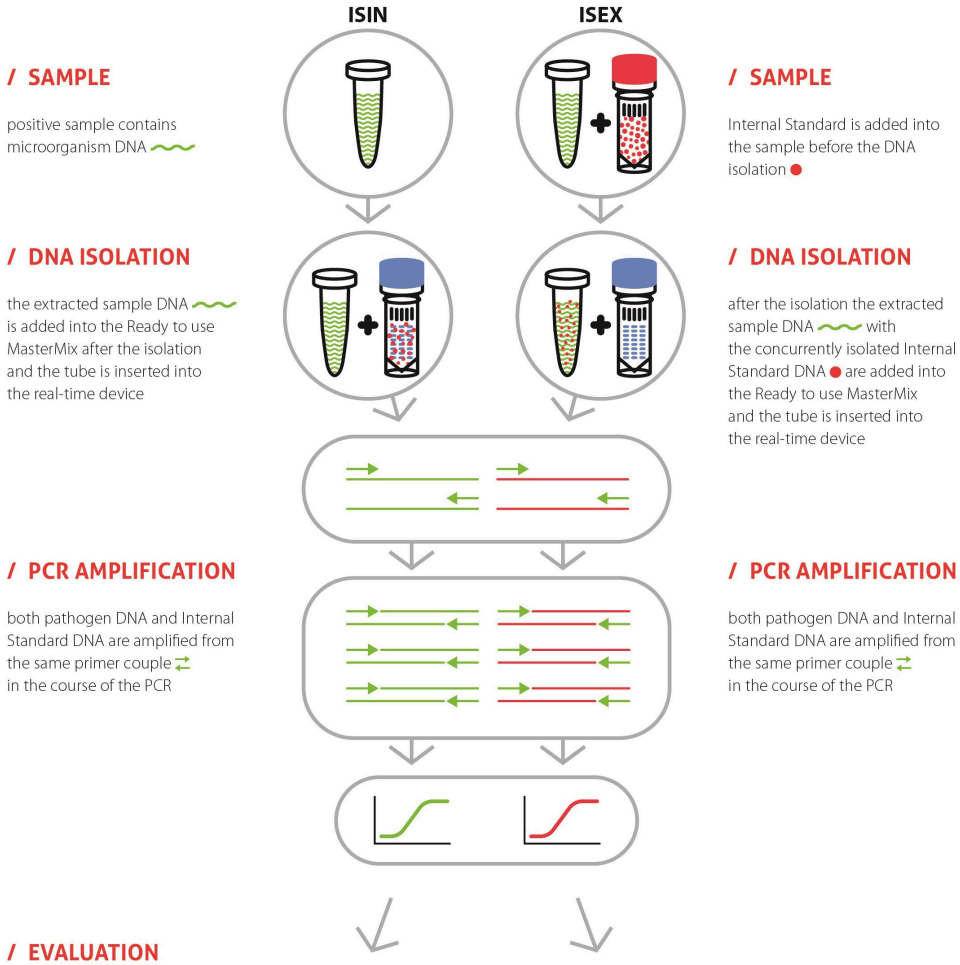
ISIN version

Internal Standard is included in the MasterMix tube. This PCR kit version enables PCR inhibition control.

ISEX version

Internal Standard is provided as independent item within the package. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



POSITIVE SAMPLE
– exponential fluorescence growth in the FAM fluorophore is evident if the detected pathogen DNA is present in the sample

QUALITY CONTROL FOR THE DIAGNOSTIC PROCESS
– exponential growth of the HEX fluorophore fluorescence, as a result of the IS amplification, controls the following:

1. Inhibition and efficiency of the PCR amplification – **ISIN** version
2. DNA extraction quality, inhibition and efficiency of the PCR amplification – **ISEX** version

USER MANUAL

SAMPLING AND SAMPLE STORAGE

For VRE detection it is possible to use whole blood (2 ml in EDTA), urine, stool (1 g) or sputum (approx. 1 ml). Samples should be transported into the laboratory within 12 to 72 hours after sampling. Store and transport samples in temperature range +2 °C to +8 °C. For long-term storage keep frozen in -85 °C to -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

When using the ISEX versions of the PCR kits the IS should be added directly into the sample at the beginning of the isolation process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IS:

Elution volume	**25 µl**	**50 µl**	**100 µl**	**200 µl**
Internal Standard	2,5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl.
It is necessary to keep all components at +2°C to +8°C during the PCR preparation.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
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.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	37 °C	2 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM+HEX+Cy5	45
	60 °C	40 s		
	72 °C	20 s		

VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

Rotor-Gene 3000/Q*
LightCycler® 480
CFX96™ /Dx Real-Time PCR Detection System
SLAN® Real-Time PCR System
LineGene 9600*

* Validation applies to a device model providing detection in the following channels: FAM, HEX and Cy5.
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.