

## GeneProof Legionella pneumophila PCR Kit



*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

REF	ISIN Version IS included in the MasterMix			ISEX Version IS supplied in a separate tube Nucleic acid extraction and PCR inhibition control		
	LP/ISIN/025 25 rxn	LP/ISIN/050 50 rxn	LP/ISIN/100 100 rxn	LP/ISEX/025 25 rxn	LP/ISEX/050 50 rxn	LP/ISEX/100 100 rxn
<b>MasterMix</b> <i>Legionella pneumophila</i>	1x750 µl	2x750 µl	4x750 µl	1x750 µl	2x750 µl	4x750 µl
<b>Positive Control</b> <i>Legionella pneumophila</i>	1x200 µl	1x200 µl	2x200 µl	1x200 µl	1x200 µl	2x200 µl
<b>Internal Standard</b> <i>Legionella pneumophila</i>	-	-	-	1x1000 µl	1x1000 µl	2x1000 µl

### STORAGE AND TRANSPORTATION CONDITIONS

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 15 repeated freezing/thawing cycles.

### TECHNICAL SPECIFICATION

<b>Target Sequence</b>	The gene encoding 16S rRNA specific for <i>L. pneumophila</i>
<b>Specificity</b>	<i>L. pneumophila</i> , 100 %
<b>Sensitivity (LoD)</b>	Reaches up to 0.578 cp/µl with the probability 95 % (on Amplirun® <i>Legionella pneumophila</i> DNA control, Vircell)
<b>Validated Specimen</b>	BAL, sputum, swab
<b>Quality Control</b>	In accordance with ISO 13485, each lot of GeneProof PCR Kit is tested against predetermined specifications to ensure consistent product quality.
<b>External Quality Assessment</b>	Regularly tested by QCMD and Instand e.V. External Quality Assessment Panels

## METHOD PRINCIPLES

The PCR kit is designed for *Legionella pneumophila* detection by the real-time Polymerase Chain Reaction (PCR) method. The *L. pneumophila* detection consists in amplification of the gene encoding 16S rRNA specific for *L. pneumophila* and in measurement of fluorescence increase. The *L. pneumophila* presence is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is either included in the reaction mix, controlling the possible inhibition of the PCR (ISIN version) or excluded, controlling also the DNA extraction process quality (ISEX version). IS positive amplification is detected in the HEX fluorophore fluorescence channel. The detection kit takes advantage of 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 by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

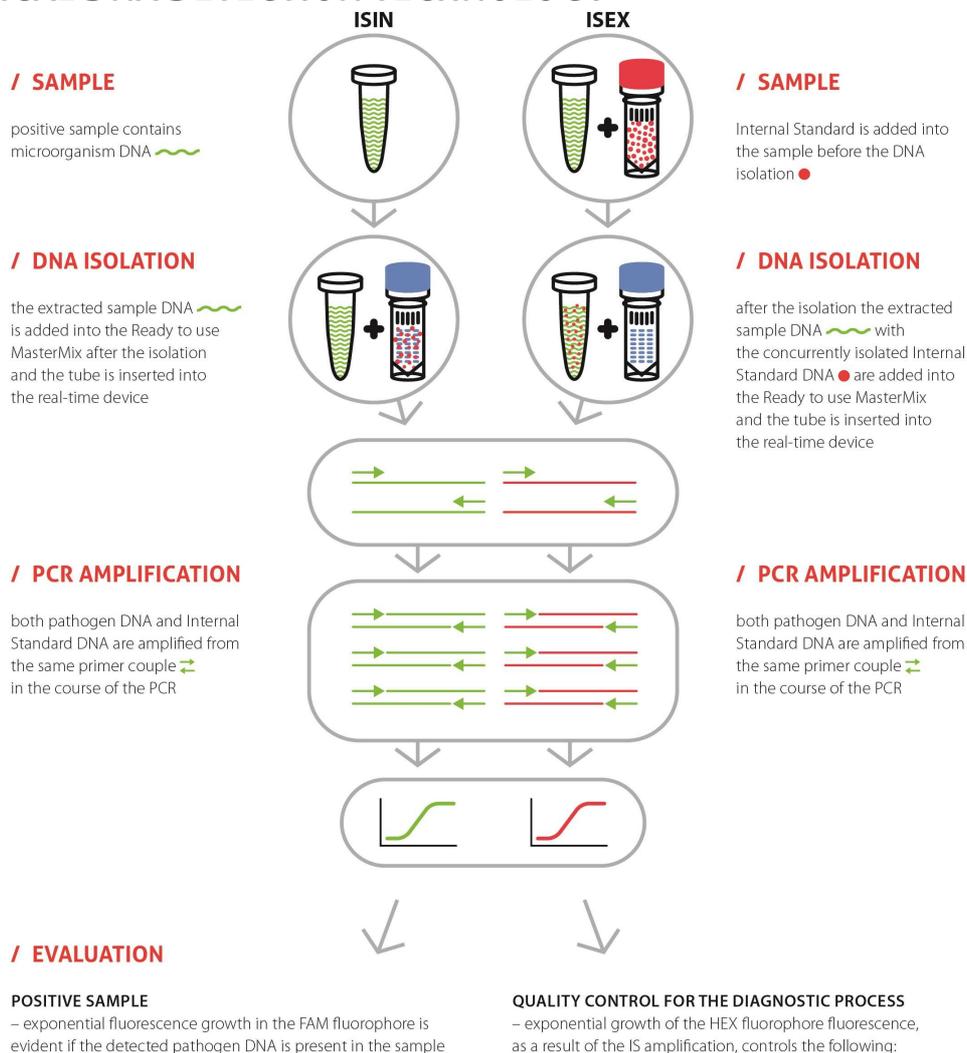
### ISIN version

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 purification process efficiency control.

## MICROBIOLOGICAL DNA DETECTION TECHNOLOGY



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

*Legionella* detection in human clinical diagnostics is feasible from sputum, bronchoalveolar lavage samples (BAL) or swabs. Sampling should be performed into sterile tubes without any transportation media and the samples should be transported to the laboratory within 24 hours at the temperature between +2 °C and +8 °C. In case of longer storage keep all samples frozen at the temperature below -20 °C.

## NUCLEIC ACID PURIFICATION

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

croBEE NA16 Nucleic Acid Extraction System

GeneProof PathogenFree DNA Isolation Kit

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		
	60 °C	40 s	FAM+HEX	45
	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:

croBEE Real-Time PCR System

Applied Biosystems 7300 / 7500 Real-Time PCR System

AriaMx Real-Time PCR System

LightCycler® 2.0 / 480

LineGene 9600 / 9600 Plus

Rotor-Gene 3000 / Q

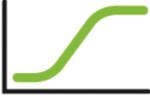
SLAN® Real-Time PCR System

Required Detection Channels FAM, HEX

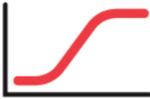
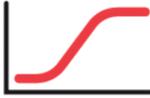
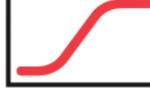
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at [support@geneproof.com](mailto:support@geneproof.com).



## CLINICAL SAMPLES ANALYSIS EVALUATION

Channel FAM	Channel HEX	Result	Interpretation	
		Valid	<i>Legionella pneumophila</i>	<b>positive</b>
		Valid	<i>Legionella pneumophila</i>	<b>positive</b>
		Valid	<i>Legionella pneumophila</i>	<b>negative</b>
		Invalid		
		Invalid		

## CONTROL ANALYSIS EVALUATION

	Channel FAM	Channel HEX	Result
Positive Control		 or 	Valid
Negative Control		 or 	Valid
Positive Control		 or 	Invalid
Negative Control		 or 	Invalid

## WARNING

A single valid package insert for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not 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 our Customer Service.

### Customer care and technical support

Tel.: +420543211679  
 Fax: +420516770824  
 email: support@geneproof.com

### Orders

Tel.: +420543211679  
 Fax: +420516770824  
 email: sales@geneproof.com

