

GeneProof®

Legionella pneumophila

PCR Kit



in vitro Diagnostics

The kit is designed for professional use in specialized clinical and research laboratories.

Kit composition

Cat. No.	Internal Standard is included in the MasterMix for inhibition control			Contains a separate tube of Internal Standard for inhibition and isolation process control		
	LP/ISIN/025 25 reactions	LP/ISIN/050 50 reactions	LP/ISIN/100 100 reactions	LP/ISEX/025 25 reactions	LP/ISEX/050 50 reactions	LP/ISEX/100 100 reactions
MASTERMIX <i>Legionella pneumophila</i>	1 x 750 µl	2 x 750 µl	4 x 750 µl	1 x 750 µl	2 x 750 µl	4 x 750 µl
POSITIVE CONTROL <i>Legionella pneumophila</i> 10 ² copies/µl	1 x 200 µl	1 x 200 µl	2 x 200 µl	1 x 200 µl	1 x 200 µl	2 x 200 µl
INTERNAL STANDARD <i>Legionella pneumophila</i>	-	-	-	1 x 1000 µl	1 x 1000 µl	2 x 1000 µl

Storage and transportation conditions

Transport the kits at temperatures ranging from -20°C to -80°C. The kit remains stable for 9 months from the date of manufacturing at the temperature of -20°C. Repeated freezing and thawing of the MasterMix, Internal Standard or the Positive control may result in lower detection quality. The manufacturer therefore recommends to aliquot the MasterMix by 30 µl directly to PCR tubes and hold in stock at -20°C. Positive control and the Internal Standard may be held in stock at 4°C.

Pathogen information

Legionellas naturally occur in water reservoirs. Human infections may proceed asymptotically, yet Legionellas may cause insignificant “flue-like” disease or even serious pneumonias (incubation time 2-10 days, fever, dry cough, chest pain and head pain, possible affection of kidneys, liver or CNS, especially a picture of multiple bronchopneumonia with microabscesses in lungs and death-rate of up to 20%). Elderly people and people with decreased immunity establish the group in danger. People usually get infected from water reservoirs, artificial water bodies, air-conditioning device water or there may occur nosocomial infections in immunosuppressed patients. Diagnostics is possible through a rather difficult cultivation or immunofluorescence, the ELISA method is able to detect a specific antigen in urine (this test is specific for serotypes and after an endured infection it remains positive for a long time). Antibody demonstration is performed by the ELISA method. Direct diagnostics by the PCR method is about as sensitive as cultivation, yet it is less demanding and much faster – this is rather significant for the subsequent therapy. It can be used mostly for fast demonstration in threatened patient groups or for a very sensitive demonstration of Legionellas in samples from the environment (water reservoirs, swabs and samples from air-conditioning units of hospital and hotel facilities). The PCR method is considered to be valuable, yet complementary method with growing significance in the human clinical diagnostics.

Method principles

The kit is designed for the detection of the *Legionella pneumophila* genomic DNA based on the amplification of the *L. pneumophila* specific sequence of the gene encoding the ribosomal 16S RNA subunit by means of the Polymerase Chain Reaction (PCR) and for measuring of the amplification product concentration growth in the course of the PCR by means of the fluorescence marked probe (real-time PCR). Legionella DNA presence in the sample is indicated by the FAM fluorophore fluorescence growth. An Internal Standard (IS) is included in the reaction mix, controlling the possible inhibition of the PCR reaction and the efficiency of the DNA isolation process. IS positive amplification is detected in the fluorescence channel for the JOE fluorophore. The detection kit takes an advantage of the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. It contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. This assures very high sensitivity of the laboratory Legionella detection in clinical material. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

GeneProof PCR kits are designed for use with real-time devices from various manufacturers.

***Legionella pneumophila* PCR Kit has been validated with the following devices:**

Rotor-Gene™ 3000 (Corbett Life Science)
Rotor-Gene™ 6000 (Corbett Life Science)
7500 Real-Time PCR System (Applied Biosystems)
LightCycler® 2.0 (Roche)
LightCycler® 480 System (Roche)
SLAN Real-time Quantitative PCR Fluorescent Detection System (Shanghai Odin Scienc & Technology Co.)

For detailed information about PCR kit use with specific devices see the Manufacturer’s web site (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, please: support@geneproof.com

Warning:

- The kit has been manufactured according to the EC Directive 98/79/EC as an *in vitro* medical diagnostic device.
- 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.

User Manual

Sampling and sample storage

Legionella detection in human clinical diagnostics is feasible from throat washing, BAL, urine, blood (preferably blood serum or plasma). Sampling of all sample types, except for blood, should be performed into sterile tubes without any transportation media and the samples should be transported within 12 hours at +4°C. It is necessary to sample up to 2ml of body fluid samples (BAL, urine, etc.) ; max. 1x1x1mm of tissue (in case of lung biopsy tests); swab or scraping on a swab "dry". Blood sampling: a sample of incoagulable peripheral blood should be sampled into EDTA and transported into the laboratory at +4 °C within 24 hours. In case of longer storage all samples should be frozen at -20°C.

DNA isolation

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

All GeneProof PCR kits include an Internal Standard (IS) providing for an effective monitoring of eventual inhibition of the PCR amplification and also of the isolation process efficiency. The Internal Standard is a precisely defined and quantified construct of a plasmid and insert, prepared by genetic engineering methods GeneProof develops and sells two basic variants of PCR kits which differ in the Internal Standard composition.

PCR Kit ISIN (Cat. No. LP/ISIN...)

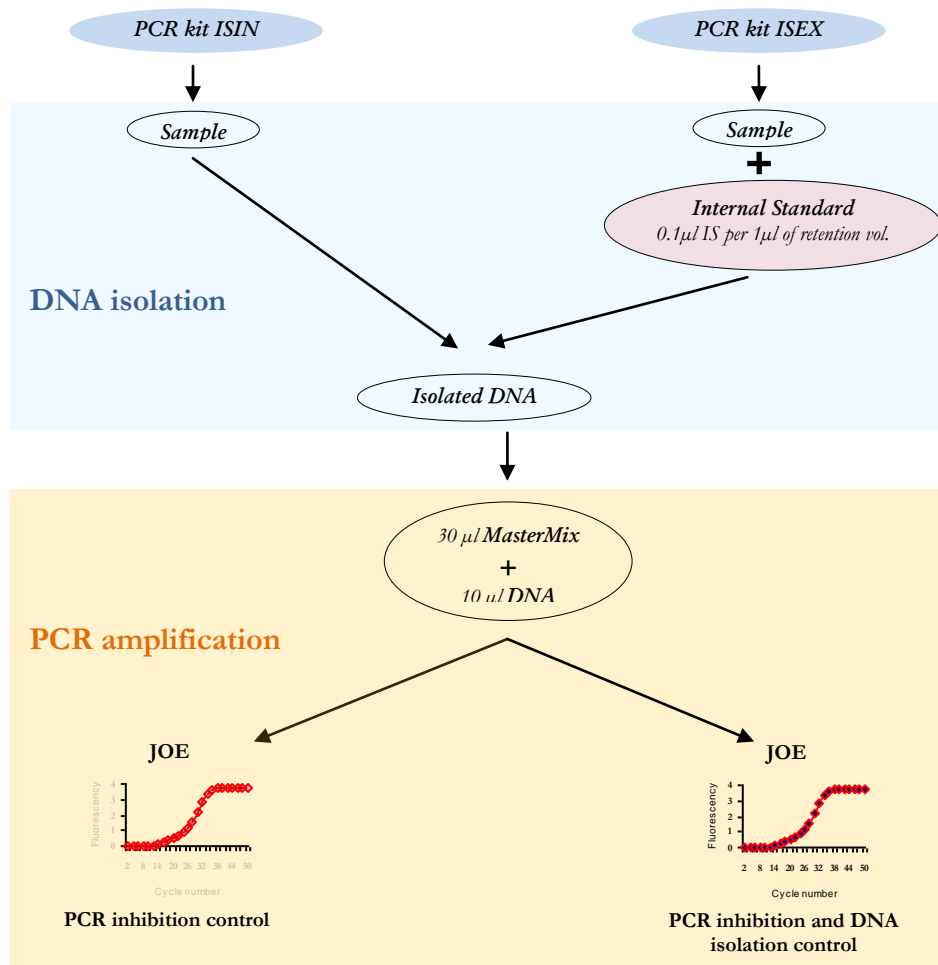
In this version of the PCR kit the Internal Standard (IS) is included directly in the MasterMix tube. This version of the kit provides **efficient control of the PCR reaction inhibition.**

PCR Kit ISEX (Cat. No. LP/ISEX...)

In this PCR kit version the Internal Standard (IS) is included in a separate tube within the package. This version of the PCR kit can be used for both **PCR reaction inhibition control** and **DNA isolation efficiency control.**

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 amplification

1. Add **30 µl of the MasterMix** and **10 µl of the DNA isolate** or **10 µl of the Positive Control** into a PCR tube. The final reaction mix volume should be 40 µl.
2. Close the tubes, centrifuge shortly, insert into the device and program according to the following table:

Amplification program:

UDG decontamination	37°C/2 min.
initial denaturation	95°C/10 min.
denaturation	95 °C/5 sec.
annealing	60°C/40 sec. - reading of the fluorescence signal
extension	72°C/20 sec.
number of cycles	45

Qualitative evaluation of detection

