



PCR-Legionella spp Plus Detection Kit

(Catalog# K1451-96; 96 Rxns; Storage at -20°C)

I. Introduction:

Waterborne pathogens and related diseases are a major public health concern worldwide, not only by the morbidity and mortality that they cause, but by the high cost that represents their prevention and treatment. These diseases are directly related to environmental deterioration and pollution. Legionella contamination in air conditioners and water supply systems poses a serious health concern. BioVision offers PCR-based detection kits intended for the specific, rapid, and reliable detection of this pathogen in a user-friendly and cost-effective format.

PCR-Legionella spp Plus Detection Kit (Legionella spp & Legionella pneumophila) is an ideal tool for a fast and reliable Amplification and Detection of specific DNA fragments from Legionella spp and/or Legionella pneumophila by the real-time PCR method (Multiplex PCR). The Kit includes all reagents required in a comfortable ready-to-use Multiplex PCR MasterMix. The optimized MasterMix contains a Buffer, dNTPs, Hot-start DNA Polymerase, DNA-free water, MgCl₂ and an Internal Amplification Control (IAC) whose detection indicates the absence of PCR inhibitors. Primers and Probes for the amplification of IAC as well as for the amplification of the target gene are included in the MasterMix. The probe for the detection of target gene is labeled with the FAM (Legionella spp) and HEX fluorochrome (L. pneumophila), whereas the probe for the detection of IAC is labeled with the CY5 fluorochrome.

Additionally, the kit includes both Positive Control and Negative Controls. The Positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a Negative control reaction should be included every time the kit is used.

II. Applications:

- An ideal tool for a fast and reliable Amplification and Detection of specific DNA fragments from Legionella spp and/or Legionella pneumophila by the real-time PCR method (Multiplex PCR).

III. Sample Type:

- Water sample

IV. Kit Contents:

| Components | K1451-96 | Part Number |
|--------------------------|----------|-------------|
| Multiplex PCR Master Mix | 2 Vials | K1451-96-1 |
| PCR Positive Control | 1 Vial | K1451-96-2 |
| PCR Negative Control | 1 Vial | K1451-96-3 |
| DNA Lysis Buffer | 2 Vials | K1451-96-4 |

V. User Supplied Reagents and Equipment:

- PCR tube or plate well
- Centrifuge
- Polycarbonate filter & DNA purification system
- Real Time Thermal Cyclers such as Agilent Mx3005P, Applied Biosystems 7300, 7500 or other cyclers.

VI. Storage Conditions and Reagent Preparation:

All the reagents are shipped in dry ice and stored at -20°C upon receipt. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 6 months.

VII. Assay Protocol:

Sample Preparation:

1. Collect 1L of the original water sample to be concentrated by filtration.
2. Filter the collected volume using a polycarbonate filter or any other compound with low capacity for adsorption of protein or DNA, with a nominal porosity of 0.45 µm or less.
3. Remove aseptically from the holder filter with sterile forceps, folded to the outside, and place into a sterile, 50 mL centrifuge tube containing 5-10 mL of diluent reagent (e.g. sterile distilled water). Optionally you can use scissors to cut the filter into several pieces.
4. Elute the filter by shaking. The shaking can be manual (2 min), or vortex (2 min), or magnetic stirrer (low revolutions), or ultrasound bath (5 min).
5. Centrifuge the centrifuge tube at 8000g for 10 minutes. Remove the supernatant using a pipette leaving 1ml of residual liquid.
6. Mix by vortex.
7. Proceed to the extraction of DNA from the Mix with the method of choice.
Note: In case DNA purification system with silica column or magnetic beads is used, please elute in 50 µl. Optionally you can use our DNA Lysis Buffer suitable for DNA extraction.

Standard Curve:

1. Prepare a suspension of an active culture of Legionella pneumophila (maximum 3 days of growth; O.D_{600nm} 0.5 to equivalent 10⁹ cfu/ml)
2. Extract the DNA with the same procedure used for the samples.
3. Determine the DNA concentration of the extract.
4. Calculate the number of genomic units (GU) of the average weight of the Legionella pneumophila genome (~3.5 Mb)
5. Prepare a bank of decimal dilutions with a dynamic range of 5 log from 10 GU/µl
6. Express the results in GU/liter of filtered water.

PCR reaction:

1. Load 5 µl of the extracted DNA samples into each PCR tube or plate well containing 15 µl of the reaction mix.
2. Load also 5 µl of the positive controls into the appropriate tubes or plate wells.
3. Place the PCR tubes or the plate into the real time thermal cycler. Set the fluorescence reading at the channels corresponding to the fluorochromes FAM, HEX and CY5.



PCR cycling conditions:

| Step | Time | Temperature |
|----------------------|------------------------------------|-------------|
| Initial Denaturation | 10 min | 95°C |
| 40 Cycles | 15 sec | 95°C |
| | 1 min | 60°C |
| Melt Analysis | Refer to instrumental instructions | |

Fluorescence measurements should be carried out at the end of each Annealing/Extension cycle at 60°C.

Analysis of results:

1. Follow instrument software instructions to generate cycle threshold (Ct) values from the acquired data. The user may also, optionally, analyze the melt profile of each reaction. The quantity of DNA target in each sample can be calculated by referring to the positive control template Ct value.
2. A result will be considered as positive whenever fluorescence corresponding to Legionella spp and Legionella pneumophila intercepts the threshold value for detector. It is recommended to analyze each fluorescence channel separately.
3. A reaction will be considered negative whenever no amplification curve is produced or fluorescence does not cross the threshold and there is an amplification curve for IAC at the expected Ct.

VIII. Related Products:

| Product Name | Cat. No. | Size |
|--|----------|----------|
| PCR-Salmonella Detection Kit | K1447 | 96 Rxns |
| PCR-Salmonella-Listeria Detection Kit | K1448 | 96 Rxns |
| PCR-Listeria monocytogenes Detection Kit | K1449 | 96 Rxns |
| PCR-Legionella spp Detection Kit | K1450 | 96 Rxns |
| PCR-STEC Detection Kit | K1452 | 96 Rxns |
| PCR-Campylobacter Detection Kit | K1453 | 96 Rxns |
| Coronavirus Rapid RT-qPCR Detection Kit | K1461 | 100 Rxns |

FOR RESEARCH USE ONLY! Not to be used on humans.