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PCR-Legionella spp Detection Kit

(Catalog# K1450-96; 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 Detection Kit is an ideal tool for a fast and reliable amplification and detection of specific DNA fragment from Legionella spp by real-time PCR method. All reagents required for qPCR are provided as a ready-to-use Master Mix. The PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and $MgCl_2$ to perform the number of reactions indicated in the kit. The PCR Master Mix also includes 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 Master Mix. The probe for the detection of target gene is labeled with the FAM (Legionella spp), whereas the probe for the detection of IAC is labeled with the JOE/HEX fluorochrome. The reaction mix does not contain ROX.

Additionally, the kit includes positive control DNA (Legionella pneumophila LPN457, 10^5 copies/ul) and negative control (Molecular biology grade Water). The positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm the 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 fragment from Legionella spp by the real-time PCR method.

III. Sample Type:

- Bacteria, Legionella spp
- Environmental and Biological Samples (water, sediments, sputum, blood, serum, and urine samples).
- Air conditioners and water supply systems

IV. Kit Contents:

Components	K1450-96	Part Number
PCR Master Mix	1 Vial	K1450-96-1
PCR Positive Control	1 Vial	K1450-96-3
PCR Negative Control	1 Vial	K1451-96-4

V. User Supplied Reagents and Equipment:

- Environmental and Biological Samples
- PCR tube
- Centrifuge
- Thermal cycler

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 12 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 or Ringe).Optionally you can use scissors to cut the filter into several pieces.
4. Elute the filter by shaking. The shaking can be manual (2 minutes), or vortex (2 minutes), or magnetic stirrer (low revolutions), or ultrasound bath (5 minutes).
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.

Standard Curve:

1. Prepare a suspension of an active culture of Legionella spp (maximum 3 days of growth; $O.D^{600}$ nm 0.5 to equivalent 10^9 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 spp genome (approximately 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 ul of the reaction mix.
2. Load 5 µl of the positive controls also 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 and JOE/HEX.

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	

Analysis of results:

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.

A result will be considered as positive whenever fluorescence corresponding to Legionella spp intercepts the threshold value for detector. It is recommended to analyze each fluorescence channel separately. 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:

- PCR Master Mixes and Kits (Cat# M1127- M1145)
- DNA Extraction (Cat# K1411-K1417); K309; K316
- Q-PCR (Cat# M1105-M1126)

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