

# Data sheet

## PRImeDETECT™ Legionella spp Plus Detection Kit (*Legionella* spp & *Legionella pneumophila*)

Cat. No: FP0025 (48 reactions)

Cat. No: FP0026 (96 reactions)

### 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. Canvax Biotech SL offers molecular microbiology PCR-based detection systems intended for the specific, rapid, and reliable detection of this pathogen in a user-friendly and cost-effective format.

**PRImeDETECT™ Legionella spp Plus Detection Kit (*Legionella* spp & *Legionella pneumophila*)** is based on amplification and detection of specific DNA fragments from *Legionella* spp and/or *Legionella pneumophila* by the real-time PCR method (multiplex PCR).

All reagents required for qPCR are provided ready to use as Multiplex PCR Master Mix (1 vial). The Multiplex PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and MgCl<sub>2</sub> to perform the number of reactions indicated in the kit. The Multiplex 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 labelled with the FAM (*Legionella* spp) and HEX fluorochrome (*L. pneumophila*), whereas the probe for the detection of IAC is labelled with the CY5 fluorochrome.

In addition, the kit includes positive control DNA and negative control. 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

### Each kit contains:

- ✓ Multiplex PCR Master Mix (1 vial)
- ✓ PCR Positive Control (1 vial)
- ✓ PCR Negative Control (1 vial)

### Shipping and Storage

**PRImeDETECT™ Legionella spp Plus Detection Kit** is shipped at ambient temperature. On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month.

### Technical features

- ✓ **Amplification limit:** *Legionella* spp: 5 UG per reaction. *L. pneumophila*: 100 UG per reaction.
- ✓ **Quantification limit:** *Legionella* spp: 50 UG/L. *L. pneumophila*: 1000 UG/L.
- ✓ **Quantification Dynamic range:** 5 logs
- ✓ **Simultaneously** detect and differentiate *Legionella* spp. and *L. pneumophila* in the same tube
- ✓ **Inclusivity:** Primers and probes have been tested satisfactorily in 14 strains of *L. pneumophila* and 30 species of the genus *Legionella*.
- ✓ **Compliance:** ISO/TS 12869:2012
- ✓ **Exclusivity:** No cross-reactions with other frequent bacterial agents have been observed.
- ✓ **Detection:** probe labelled with fluorescent dyes—*Legionella*: FAM-BHQ1a; *L. pneumophila*: HEX-BHQ1; IAC:CY5-BHQ2.
- ✓ **Thermal cycler:** Agilent Mx3005P, Applied Biosystems 7300, 7500 and other cyclers.

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## 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  $\mu\text{m}$  or less (ISO/TS 12869:2012).
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 8000  $\times$  g for 10 minutes. Remove the supernatant using a pipette leaving 1ml of residual liquid.

Note: If the objective of the analysis is the quantification of viable, apply the PMA by this step and before the extraction and purification of DNA.

6. Mix by vortex.
7. Proceed to the extraction of DNA from the Mix with the method of choice.

Note: In case of using the DNA purification system with silica column or magnetic beads (for example Maxwell RSC from Promega) elute in 50  $\mu\text{l}$ .

### Standard Curve (ISO / TS: 12869: 2012).

1. Prepare a suspension of an active culture of *Legionella pneumophila* (maximum 3 days of growth; O.D<sub>600 nm</sub> 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 pneumophila* genome (approximately 3.5 Mb)
5. Prepare a bank of decimal dilutions with a dynamic range of 5 log from 10 GU/  $\mu\text{l}$
6. Express the results in GU / liter of filtered water.

### PCR reaction:

Load 5  $\mu\text{l}$  of the extracted DNA samples into each PCR tube or plate well containing 15  $\mu\text{l}$  of the reaction mix. Load also 5  $\mu\text{l}$  of the positive controls into the appropriate tubes or plate wells.

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
<b>40 Cycles:</b>	15 sec 1 min	95°C 60°C
Melt analysis	Refer to instrument instructions	

\*Fluorescence measurements should be carried out during Step 2 at the end of each Annealing/Extension cycle at 60 °C.

## 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* and *Legionella pneumophila* 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.

## PRECAUTIONS

- ✓ **Good Laboratory Practice** must be observed in order to obtain reliable results with this technique. The high sensitivity of this test requires extreme care to maintain the purity of all reagents.
- ✓ Nucleic acids are very sensitive to degradation by nucleases, which are present in human skin and in surfaces that have been in contact with human skin. Wash surfaces with appropriate reagents, use powder-free examination gloves and a lab coat throughout the whole test. Wash hands thoroughly after performing the test.
- ✓ This test has been validated by using the reagents provided with **PRImeDETECT™ Legionella spp Plus Detection Kit**. The use of other amplification methods or any change in the protocol may render false results. **DO NOT INTERCHANGE COMPONENTS** from different lots.
- ✓ Do not use **PRImeDETECT™ Legionella spp Plus Detection Kit** after expiry or best before date. Store this product at the indicated temperature and conditions.
- ✓ The use of this product is limited to qualified personnel experienced in DNA extraction and amplification techniques.
- ✓ **Not For Medical Diagnostic Use.**