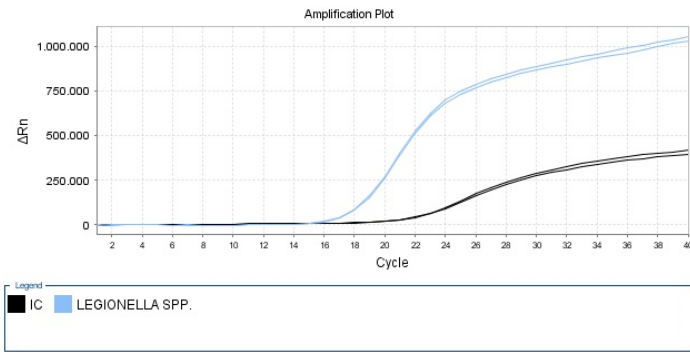


REAL TIME DETECTION KIT

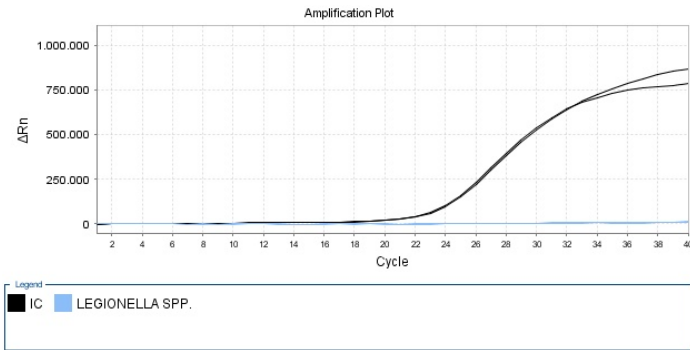
Legionella spp.

Ref: BIOPFS-0014

Positive Control –



Negative Control –



12. SPECIFICITY/INCLUSIVITY

- 100 % Exclusivity, determined using 30 strains of closely related organisms or occurring in the same habitat.
- 100 % Inclusivity, determined in 30 strains of *Legionella* spp., including *Legionella micdadei* and different serogroups of *Legionella pneumophila*.

13. SENSITIVITY

The REAL TIME DETECTION KIT *Legionella* spp. detects 10^3 - 10^4 cells/L after water concentration. This kit has a reaction sensitivity of 5 pg of target DNA.



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BPMR is certified ISO 9001:2015

1. PRODUCT DESCRIPTION

Pathogen detection kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific pathogen. The assay is based on 5' nuclease real time PCR reactions to amplify a unique genomic sequence in the target microorganism.

2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence in a reaction containing among other components, a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. The DNA molecule denatures when this solution is heated, separating into two strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands and the DNA polymerase synthesizes a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence (amplicons). When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target amplicons. In Real Time PCR, the signal is measured in each cycle, using in most cases specific fluorescent probes. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus the number of cycles, allowing the determination of the presence or absence of the target organism.

3. PATHOGEN DESCRIPTION

There were around 60 recognized species and more than 60 serogroups of *Legionella* spp.. Legionnaires' disease is the most severe form of infection, which includes pneumonia, and the fatality rate can approach 50% in immunocompromised patients. Outbreaks of Legionnaires have been worldwide traced to a wide variety of environmental water sources such as cooling towers, hot tubs, showerheads, whirlpools, spas, and public fountains. Over the last few years, the reported incidence of legionellosis has steadily increased. A commonly used method for environmental surveillance of *Legionella* spp. is the standard culture technique, but it has several limitations as it requires selective media and prolonged incubation periods. Recently, rapid and sensitive alternative methods have been found to be attractive alternatives to the conventional culture method for the detection of slow-growing and fastidious bacteria such as *Legionella* spp. These methods are PCR-based methods, in particular real-time PCR-based, that allow the rapid detection of *Legionella* spp. in water samples.

4. INTERNAL CONTROL (IC)

Pathogen detection kits include an Internal Control (IC) in the master mix. This control is amplified at the same time as the target DNA sequence, but using a different set of primers and a probe labeled with a different fluorophore. The inclusion of the IC in each reaction allows the evaluation of PCR inhibitors in negative results.

5. APPLICATION

REAL TIME DETECTION KIT *Legionella* spp. is intended for the rapid detection of *Legionella* spp. in environmental swab samples and water samples following concentration by membrane filtration and DNA extraction. It can also be used with other environmental samples. The kit was validated in the instruments ABI PRISM® 7500 Fast and ThermoScientific® PikoReal. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Research use only.

The procedure includes the following main steps:



6. CONTENTS AND STORAGE

The kit contains reagents for 100 assays

| CONTENTS | UNITS | COMPOSITION |
|------------------------------|------------------------|-------------------------------|
| Master Mix (blue cap) | ● 2 tubes (2 x 840 µl) | Buffer, dNTPs, DNA polymerase |
| Assay Mix (brown cap) | ● 1 tube (1 x 210 µl) | Primers and Probes |
| Negative Control (clear cap) | ○ 1 tube (1 x 70 µl) | Nuclease-free water |
| Positive Control (red cap) | ● 1 tube (1 x 70 µl) | Target DNA |

Store all contents at -20°C and protect them from light as excessive exposure to light may affect the fluorescent probes. Minimize freeze-thaw cycles. Reagents stored as recommended may be used until the expiration date indicated in the tube.

7. MATERIAL REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease-free filter tips
- Real-time PCR instrument
- Tubes/Strips/Multiwell plates and accessories specific for each Instrument
- Lysis buffer / DNA extraction kit (example: BIOPEXT-0400)

8. PRECAUTIONS AND RECOMMENDATIONS

Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. The user should always pay attention to the following:

- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

9. QUALITY CONTROL

In accordance with BPRM's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

10. PROCEDURE

10.1 Sample preparation

Water samples shall be concentrated according to ISO 11731.

10.2 DNA extraction

1. Resuspended the filtrate in 1 mL of 0.9% NaCl solution or PBS and centrifuge at 10,000 - 12,000 g for 5 min.
2. Discard all the supernatant.
3. Add 100-200 µL of lysis buffer to the pellet and follow the instructions of BIOPREMIER DNA Rapid Extraction Buffer (ref: BIOPEXT-0400) (not included) or other kit or suitable protocol for DNA extraction.

10.3 PCR preparation

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly and centrifuge briefly
2. Prepare the reactions, as described below:

| CONTENTS | N° OF SAMPLES | |
|---------------------|---------------|---------------|
| | 1 | 10 (10 + 1) |
| Master Mix ● | 16 µl | 176 µl |
| Assay Mix ● | 2 µl | 22 µl |
| Total Volume | 18 µl | 198 µl |

Note: Prepare the PCR reaction for each sample, or in the alternative, prepare a Master Mix for the total number of reactions plus 10%, to cover pipetting losses (e.g. for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 18 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 2 µL of Negative Control tube (Clear Cap); Pipette 2 µL of DNA sample per well; and for the positive control, pipette 2 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. Place the reactions into the Real Time PCR instrument.

B – Program set up

Prepare the Real-Time PCR instrument according to the following temperature/time program:

| PHASE | STEP | TEMPERATURE | TIME | ACQUISITION |
|---------------------------|--------|-------------|---------|-------------|
| Holding Stage | Step 1 | 50°C | 2 min. | No |
| | Step 2 | 95°C | 5 min. | No |
| Amplification - 40 cycles | Step 1 | 95°C | 30 sec. | No |
| | Step 2 | 56°C | 30 sec. | Yes |
| | Step 3 | 72°C | 30 sec. | No |

| TARGET | CHANNELS |
|-------------------------------------|----------|
| Detection of <i>Legionella</i> spp. | FAM |
| Detection of Internal Control (IC) | ROX |

11. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.

Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Assessment of sample results should be performed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted. Interpretation of PCR data:

a) Controls

To validate the assay, the controls must have the following results:

| | <i>Legionella</i> spp. DETECTION FAM | IC DETECTION ROX |
|------------------|-----------------------------------------|---------------------|
| Negative Control | Negative | Positive |
| Positive Control | Positive | Positive |

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples

Interpretation of sample results is summarized in the following table:

| <i>Legionella</i> spp. DETECTION FAM | IC DETECTION ROX | INTERPRETATION |
|-----------------------------------------|---------------------|----------------------------------------------------------------|
| Positive | Positive/Negative** | DNA detected, sample Positive for <i>Legionella</i> spp. |
| Negative | Positive | No DNA detected, sample Negative for <i>L. Legionella</i> spp. |
| Negative | Negative | Invalid Result* |

*When both *Legionella* spp. and IC detection are Negative, this means the presence of PCR inhibitors in the sample. Dilute the DNA sample (10-fold dilution) or perform another DNA extraction and repeat qPCR.

** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.