

TECHNICAL SHEET

***qualyfast*<sup>®</sup> Legionella**

qPCR detection Kit of Legionella species and L. pneumophila  
with Inhibition Amplification Control

## INTENDED USE

---

**qualyfast<sup>®</sup> Legionella** product is rapid, sensitive, specific and reproducible kit for detection, discrimination and quantification of *Legionella* species and *Legionella pneumophila* from water. *Legionella* bacteria are ubiquitous organisms typical of freshwater environments. *L. pneumophila* has been recovered from a wide range of both human-made and natural aquatic habitats, from lakes and streams to air-conditioning cooling towers, fountains and spa baths. *Legionella* bacteria are not free-living aquatic bacteria; rather, they parasitize or form a commensal relationship with free-living, freshwater and soil amoebae. When inhaled by humans it can replicate within alveolar macrophages and cause a severe pneumonia, Legionnaires disease.

## PRINCIPLE

---

**qualyfast<sup>®</sup> Legionella** kit provides:

- amplification of specific DNA sequences belonging to the genus *Legionella* species and the species *L. pneumophila*;
- simultaneous co-amplification of a IAC (Inhibition Amplification Control). This approach is used to highlight a possible effect of inhibition in the DNA from the sample.

Real Time PCR of specific sequences of *Legionella* species and *Legionella pneumophila* is performed. The *Legionella* spp. detection is performed on FAM channel. **qualyfast<sup>®</sup> Legionella** is integrated with an Inhibition Amplification Control (IAC) detecting by measuring the VIC/HEX fluorescence. The discrimination of *Legionella pneumophila* is performed on CY5 channel. The kit is compatible with the principal real-time PCR instruments. The use of probes and oligonucleotides mixture allows to monitor in real time the reaction in progress following the fluorescence increase for each sample.

The thermal profile is:

1 cycle: 95 °C for 10 minutes;

50 cycles: 95 °C for 15 seconds; 60 °C for 30 seconds (with acquisition of the fluorescence data).

The same profile is used for all Bioside's products.

## MATERIALS PROVIDED

---

All the reagents are pre-dosed and lyophilized in the reaction tubes, packed in strips of 8. Each kit is also provided of DNA free solution tube and one strip of 8 reaction tubes of positive controls ready to use. The provided quantity of reagents allows to carry out 24, 48 or 96 reactions (strips of 8 reactions each) as indicated in the table below.

Catalog Number	Number of reactions	Plastics type
A1.01013	24 (3 strips of 8 reactions each)	0,1 ml clear strip
A1.11013	24 (3 strips of 8 reactions each)	0,2 ml clear strip

A1.21013	24 (3 strips of 8 reactions each)	0,1 ml white strip
A2.01013	48 (6 strips of 8 reactions each)	0,1 ml clear strip
A2.11013	48 (6 strips of 8 reactions each)	0,2 ml clear strip
A2.21013	48 (6 strips of 8 reactions each)	0,1 ml white strip
A3.01013	96 (12 strips of 8 reactions each)	0,1 ml clear strip
A3.11013	96 (12 strips of 8 reactions each)	0,2 ml clear strip
A3.21013	96 (12 strips of 8 reactions each)	0,1 ml white strip

## MATERIALS REQUIRED BUT NOT PROVIDED

---

- 0,1 / 0,2 ml microcentrifuge
- Biological cabinet
- Filtered tips (aerosol barrier)
- Pipettor (capacity 2-20 µl)
- 0,1 / 0,2 ml tube rack
- Disposable gloves, powderless
- Real Time PCR Thermalcycler that allows the detection of FAM, HEX/VIC and CY5 fluorophores

## ANALYSIS PROCEDURE

---

Start the software of the real time PCR Thermalcycler and the real time PCR Thermalcycler. Program the instrument with the thermal profile and fluorophores indicated above. The reaction volume is 15 µl.

For some real-time PCR instruments, the type of the probe quencher as well as the usage of a passive reference dye has to be determined. The **qualyfast® Legionella** contains probes with a non fluorescent quencher and passive reference is not present; if necessary specify on the plate setup.

While preparing amplification experiment always use one Positive Control tube provided with the kit. Prepare a number of reaction tubes ready to use corresponding to the number of samples to test and add one tube as negative control. Aliquot 15 µl of extracted DNA samples (if necessary, at the dilution level indicated in the DNA extraction manual) in each reaction tube, and then add 15 µl of DNA free solution in the tube selected as negative control. At last, add 15 µl of DNA free solution in the Positive Control tube. Close and spin the tubes in order to avoid bubbles.

It is recommended not to leave the reconstituted lyophilized at room temperature for more than 10 minutes.

After arranging the tubes in the real time PCR Thermalcycler, start the analysis run.

At the end of the analysis run, analyze data for each well through the Thermalcycler software, according to the instructions reported in the user manual.

The aim is to verify the presence of the *Legionella* spp. and *L. pneumophila*.

Each well corresponding to a sample must show a fluorescence in HEX/VIC for IAC and a fluorescence in FAM and CY5 in the *L. pneumophila* positive wells or only in FAM in the *Legionella* spp. positive wells.

The possible results obtained are the following:

1. Increase of the FAM fluorescence during the amplification of the *Legionella* spp. target and absence of the CY5 fluorescence during the amplification of the *L. pneumophila* target:  
presence of *Legionella* spp. genome in the sample.
2. Increase of the FAM fluorescence during the amplification of the *Legionella* spp. target and increase of the CY5 fluorescence during the amplification of the *L. pneumophila* target:  
presence of *L. pneumophila* genome in the sample.
3. Absence of the FAM fluorescence during the amplification of the *Legionella* spp. target, absence of the CY5 fluorescence during the amplification of the *L. pneumophila* target and increase of the HEX/VIC fluorescence during the amplification of the IAC:  
the samples is negative for *Legionella*, were extracted and amplified correctly.
4. Absence of the FAM fluorescence during the amplification of the *Legionella* spp. target, absence of the CY5 fluorescence during the amplification of the *L. pneumophila* target and increase of the HEX/VIC fluorescence over 40 cycle during the amplification of the IAC:  
reduced quality/quantity of the analyzed samples, which must be extracted again.
5. Absence of the HEX/VIC fluorescence during the amplification of the IAC, absence of FAM fluorescence during the amplification of the *Legionella* spp. target and absence of the CY5 fluorescence during the amplification of the *L. pneumophila* target:  
reduced quality/quantity of the analyzed sample, which must be extracted again.

## ACCESSORY PRODUCTS

---

- Bioside DNA Extraction Kit I for the rapid extraction and purification of DNA from clear water;
- Bioside DNA Extraction Kit II for the rapid extraction and purification of DNA from dirty water;
- Bioside kit of 4 quantified standard (25-25000 GU) ready to use to perform a quantitative detection;
- Bioside kit of positive controls ready to use to perform a qualitative detection;
- Bioside free DNA inactivator kit for elimination of free DNA contaminating samples.

## WARNINGS AND PRECAUTIONS

---

For professional use only.

Products are not intended for human, animal or therapeutic use but for laboratory, diagnostic and/or research.

These products do not contain any dangerous substances in concentrations >1%.

All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately.

Aseptic technique and usual precautions for handling the bacterial group studied, should be observed.

Do not use the reagents behind expiry date.

No warranty is guaranteed for products beyond their listed expiry date. No warranty is applicable unless all product components are stored in accordance with instructions for use.

Bioside offers to customers technical support and training.

## STORAGE AND SHELF-LIFE

---

The kit is ready-to-use.

Store the products in its box at room temperature until the expiry date indicate in the label. Store the tubes in their aluminum bag with their desiccant.

## SPECIFICATIONS

---

The results are expressed as presence/absence for qualitative analysis. The results are expressed in GU for quantitative analysis.

Microorganisms tested for the verification of inclusivity are:

*L. anisa*, *L. birminghamensis*, *L. bozemanii* 1 and 2, *L. cherrii*, *L. cincinnatiensis*, *L. dumoffii*, *L. Erythra* 2, *L. feeleii* 1 and 2, *L. gormanii*, *L. hackeliae*, *L. jordan*, *L. lansingensis*, *L. longbeachae* 1 and 2, *L. maceachernii*, *L. micdadei*, *L. oakridgensis*, *L. parisiensis*, *L. pneumophila* 1 to 15, *L. sainthelensi* 1 and 2, *L. tucsonensis*, *L. wadsworthii*.

Microorganisms tested for the verification of exclusivity are:

*Aeromonas hydrophila*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Burkholderia cepacia*, *Clostridium*, *Enterobacter aerogenes*, *Escherichia coli*, *Flavobacterium*, *Klebsiella oxytoca*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Serratia marcescens*, *Stenotrophomonas maltophilia*.

Microorganisms tested for the verification of exclusivity (only for *Legionella pneumophila* test) are:

*L. anisa*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. longbeachae*, *L. micdadei*, *L. parisiensis*, *L. tucsonensis*.

The limit of quantification is 25 GU.

The limit of determination is 5 GU.

## REFERENCES

---

- Anonymous. NF T90-471 Qualité de l'eau - Détection et quantification des Legionella et/ou Legionella pneumophila par concentration et amplification génique par réaction de polymérisation en chaîne en temps réel (RT - PCR). Association française de normalization; 2010. <http://boutique afnor.org>.

- Anonymous. ISO/TS 12869, «Water quality e detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR)». International Organization for Standardization; 2012.
- Anonymous. NF148 - NF Mark VALIDATION Validation protocol for commercial methods of detection and quantification of Legionella and Legionella pneumophila by concentration and gene amplification by polymerase chain reaction (PCR). Revision 2 – Adopted by AFNOR Certification on 27 May; 2013.

## PACKAGING SYMBOL

---



Catalogue number



Batch code



Temperature limitation



Keep away from sunlight



Keep dry, keep away from rain



Use by date



Manufacturer



Do not use if package is damaged



This way up