

TECHNICAL SHEET

***qualyfast*[®] Legionella**

Quantified Standards

Quantified Standards Kit for quantification of Legionella species

INTENDED USE

qualyfast® Legionella Quantified Standards product is a set of 4 ready to use quantified standards in Genomic Units (GU), for quantification of *Legionella* species, directly calibrated with the standard reference Material of *Legionella pneumophila* DNA - Certified Genome Units (GU) number.

MATERIALS PROVIDED

The kit is formed of 4 different quantified Standards, each packed in strip of 8. All the reagents are pre-dosed and lyophilized in the reaction tubes. Each kit is also provided of DNA free solution tube. The provided quantity of reagents allows to carry out 8 quantified analysis sessions.

Catalog Number	Number of reactions	Plastics type
A5.01013	24 (4 strips of 8 reactions each)	0,1 ml clear strip
A5.11013	24 (4 strips of 8 reactions each)	0,2 ml clear strip
A5.21013	24 (4 strips of 8 reactions each)	0,1 ml white strip

MATERIALS REQUIRED BUT NOT PROVIDED

qualyfast® Legionella Quantified Standards can only be used in combination with **qualyfast® Legionella** kit.

- 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 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 quantity of the standards is indicated in the Annex 1. Insert the quantity of each standard in the real time PCR instrument software in order to obtain the quantity in GU of each DNA extracted sample. The reaction volume is 15 µl.

While preparing amplification experiment always use all the four quantified standards tubes provided with the kit (QS1, QS2, QS3 and QS4). 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 in each

reaction tube, 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 quantified standards tubes (QS1, QS2, QS3, QS4). Always use all four standards provided in the kit. Spin the tubes in order to avoid bubbles.

It is recommended not to leave the reconstituted tubes 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 quantity in GU of the *Legionella* spp.

Each well corresponding to a standard must show a fluorescence in FAM during the amplification of *Legionella* spp. Target. Verify that the slope lies between $-4,115$ and $-2,839$ corresponding to amplification efficiency with a value between 75 % and 125 %.

ACCESSORY PRODUCTS

- Bioside **qualyfast**[®] DNA Extraction Kit I for the rapid extraction and purification of DNA from clear water and swabs;
- Bioside **qualyfast**[®] DNA Extraction Kit II for the rapid extraction and purification of DNA from dirty water;
- Bioside **qualyfast**[®] Legionella kit for detection of Legionella spp. and L. pneumophila.

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 in GU for quantitative analysis.

Microorganisms tested for the verification of inclusivity are:

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

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



Consult instructions for use



Caution, consult accompanying documents



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