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cleanpid®
EASY PURIFICATION

Cleanpid® Easy Purification of Legionella

Catalog number:

611-10-00

(50 tests)

Package insert

Separation method based on *Legionella* binding magnetic beads for the immunocapture and purification of *Legionella sp* in water samples.

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FAST DETECTION FOR LIFE

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I. INTRODUCTION

Cleanpid® Easy Purification kit for *Legionella* (Cat. No. 611-10-00) is a simple and rapid method for the separation and purification of *Legionella sp* in potable, natural and industrial water. The method is based on *Legionella* binding magnetic beads for the immunocapture of *Legionella* cells, following the pre-concentration of a sample.

II. THE Cleanpid® EASY PURIFICATION TECHNOLOGY

Original water sample is concentrated by filtration or similar, and the retentate is eluted (fig1). A 1 ml-portion of this prepared sample is dispensed into the capture tube.

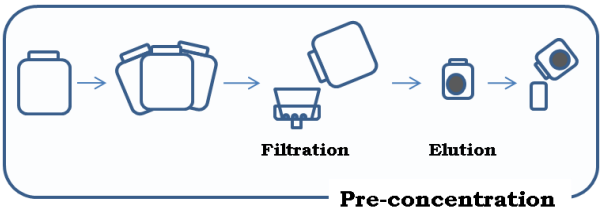


Fig 1 Obtaining prepared sample

A suspension of *Legionella* binding magnetic beads is added (fig 2). *Legionella* cells present in the prepared sample will bind to the antibodies immobilized onto the surface of the beads, to form bacteria/bead complexes. As these complexes can be separated by a *magnet*, they can be easily washed and resuspended.

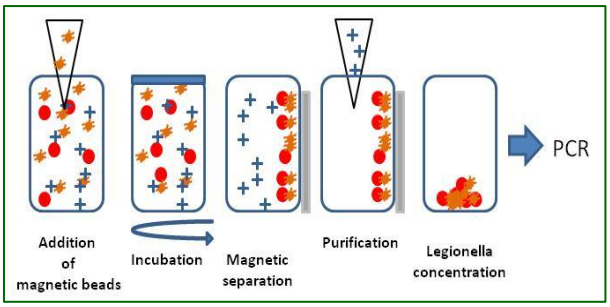


Fig 2 Main steps of the purification

After washing step, the captured *Legionella* cells can be analyzed by PCR methods.

III. KIT REAGENTS AND COMPONENTS

The kit (fig 3) comprises the reference **611-10-00 (50 tests)** and the reference **611-MRC-10** (Magnetic Particle concentrator). The elements are listed in the Table I.

Fig 3 611-10-00 (50 tests) and 611-MRC-10 (Magnetic Particle Concentrator)

Table I Elements supplied for the references 611-10-00 and 611-MRC-10

Reference 611-10-00 (50 tests)		
Reagent	**ID**	**Quantity**
Capture reagent (immunomagnetic particles)	**C1**	1 vial (1 mL)
Working buffer	**C2**	1 bottle (175 mL)
Reference 611-MRC (*)		
Component	**ID**	**Quantity**
Magnetic holder for ten tubes	**10**	1

(*) customizable device can be supplied to suit end-user´s requirements

IV. SHELF LIFE AND STORAGE

The kit must be stored between +2°C and +8°C, preferably at +4°C. The expiry time, properly stored, is 6 months from the manufacturing date. **Do not freeze**. The kit should be stored upright to always keep beads within the buffer. After use, all reagents should be rapidly stored at +2°C and +8°C, preferably at +4°C.

V. MATERIAL REQUIRED BUT NOT SUPPLIED

- Elements for filtration: filtration device, container for the filter elution, sterile membrane filter, to use with filtration system, and diluent.
- Container for residue. Eppendorf tubes.
- Pipettes of 10-100µl and 100-1000µl.
- Orbital shaker, rocker or rotator

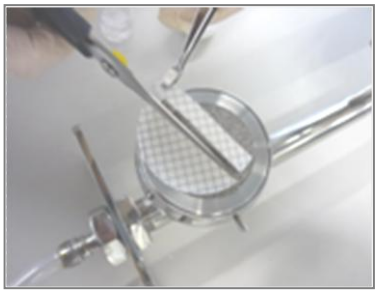
VI. PRECAUTIONS AND RECOMMENDATIONS FOR BEST RESULTS

- The product is safe under normal use. Avoid contact with eyes.
- The products are stable and unlikely to react in a hazardous manner under normal conditions of use. The product should be disposed of according to local regulations. Dispose of empty containers through the process of waste disposal.
- Do not use reagents after their expiry date.
- Shake reagent C1 by repeated gently pipetting just before use to ensure homogeneity.

VII. PROTOCOL

A. Sample preparation

1. Collect the volume of the original water sample to be concentrated (e.g. by filtration).
2. Add preferably 5 ml of the diluent reagent in a flask.
3. 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 (ISO/TS 12869:2012).
4. Carefully take the filter and deposit it in the flask with the diluent .Optionally you can use scissors to cut the filter into several pieces.



5. 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).

The eluted sample is called the concentrated sample

B. Binding using Cleanpid® *Legionella* Fast Purification Kit

This procedure describes the *Legionella* capture and concentration starting from 1 ml of concentrated sample

1. Add 1 ml of the concentrated sample to the capture tube (eppendorf)
2. Open the vial of C1 and shake gently by repeated pipetting until a completely homogeneous suspension is obtained.
3. Add 20 µl of C1 on the sample contained in the capture tube and mix by repeated pipetting. Close the lid of the eppendorf tube.
4. Incubate for 60 min at room temperature under gently agitation (approx.10-20 rpm)
5. Place the capture tube on the magnetic rack for 3 min (until all magnetic beads are retained forming a ring inside the tube and the supernatant has cleared up).
6. Discard supernatant with pipette without disturbing the retained pellet of magnetic beads

Note: Bacteria are now concentrated in the pellet

C. Washing using Cleanpid® *Legionella* Fast Purification Kit

1. Add 1 ml of C2 on the bead pellet, get the tube from the magnetic rack and then resuspend the bead pellet by repeated gently pipetting.

2. Place the tube again on the magnetic rack for 3 min (until all magnetic beads are retained forming a ring inside the tube and the supernatant has cleared up), as above.

3. Discard supernatant with pipette without disturbing the retained pellet of magnetic beads

4. Repeat points 1, 2 & 3 (of this section) two more times

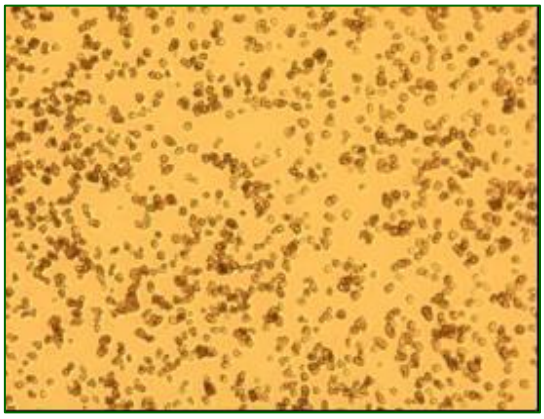
5. Resuspend the bead pellet in 100 µl of C2 by gently repeated pipetting

For PCR:

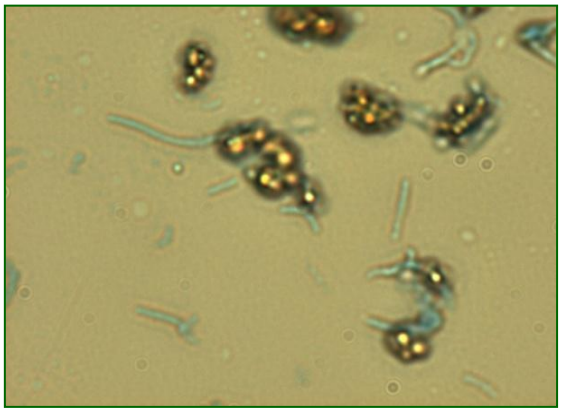
1. Apply freeze-thaw **protocol to extract DNA** starting from the resuspended pellet

2. Separate the magnetic particles by a magnet for 3 min (until supernatant has cleared up)

3. Transfer the supernatant in a new tube. Proceed with your usual **protocol for PCR**.



Magnetic beads suspension



Legionella being captured by magnetic beads

VIII. REFERENCES

1. International Organization for Standardization. 1998ISO 11731:1998. **Water quality - Detection and enumeration of *Legionella***.

2. International Organization for Standardization. 2004. ISO 11731-2:2004. **Water quality - Detection and enumeration of *Legionella*** -- Part 2: Direct membrane filtration method for waters with low bacterial counts. International Organization for Standardization, Geneva, Switzerland.

3. "**Use of immunomagnetic capture to remove interferences on *Legionella* detection by PCR and PMA-PCR"**. Inmaculada Solís, Marta Gallén, Miguel Martínez, Guillermo Rodríguez. Oral Comunication, VI National Congress on Legionella and Environmental Quality, Terrassa, Spain 12/02/2015.

Notice to purchaser: Use this product only for environmental testing

Code: **Lot number:** **Expiry date:**	For **technical assistance** please contact: Biótica, Bioquímica Analítica, S.L. Science and Technology Park, Jaume I University Building Espaitec 2, ground floor, lab 2 E12071 – Castellón, Spain www.biotica.es info@biotica.es Tel.: +34 964108131 Fax: +34 964737790	biótica® FAST DETECTION FOR LIFE